Published in final edited form as:

Vet Ophthalmol. 2014 July ; 17(0 0): 107–116. doi:10.1111/vop.12166.

The location of sites and effect of semiconductor diode transscleral cyclophotocoagulation on the buphthalmic equine globe

Anne J. Gemensky-Metzler⁺, David A. Wilkie⁺, Steven E. Weisbrode^{*}, and Sonia E. Kuhn⁺⁺

⁺Department of Veterinary Clinical Sciences, The Ohio State University College of Veterinary Medicine, Columbus, OH 43210, USA

^{*}Department of Veterinary Biosciences, The Ohio State University College of Veterinary Medicine, Columbus, OH 43210, USA

⁺⁺Department of Small Animal Clinical Sciences, University of Tennessee College of Veterinary Medicine, Knoxville, TN 37996, USA

Abstract

Purpose—To determine appropriate location and energy settings for transscleral cyclophotocoagulation (TSCPC) for buphthalmic equine globes.

Animals—Eleven horses with a buphthalmic eye blinded by glaucoma presented for enucleation.

Methods—Globe and corneal dimensions were measured via ultrasonography and calipers and TSCPC was performed under general anesthesia immediately prior to enucleation. Part 1: In 9 globes, sixty sites were lasered 4 mm posterior to the limbus in the dorsotemporal and ventrotemporal quadrants at settings of 1500 milliwatts and 1500 milliseconds. Globes were processed and sectioned sagittally over the temporal aspect in two blocks, each with 5 histologic sections examined by light microscopy. A digital imaging system was used to determine the location and length of the pars plicata on one slide from each block. Part 2: Based on results in Part 1, two globes were measured and lasered using the same time and energy settings at the following distances posterior to the limbus: 8 mm dorsally, 6 mm dorsotemporally, 5 mm ventrotemporally, 5 mm ventrally.

Results—Globe and corneal dimensions exceeded normal values in all globes. Part 1: In all 9 globes photocoagulation affected the anterior ciliary processes and iris base and in 8/9 coagulation of the pectinate ligaments was noted. Part 2: In both globes, coagulation was confined to the pars plicata.

Conclusions—The previously recommended TSCPC sites are located too far anteriorly for a buphthalmic globe. Buphthalmic equine globes should have TSCPC performed at the following distances posterior to the limbus: 6–8 mm dorsally, 5–6 mm dorsotemporally, 4–5 mm ventrotemporally and 4–5 mm ventrally.

Keywords

equine; glaucoma; buphthalmic; semiconductor diode laser; transscleral cyclophotocoagulation; histology

Introduction

Glaucoma is defined as an increase in intraocular pressure (IOP) due to obstruction of aqueous humor outflow that results in damage to the retina and the optic nerve and ultimately, vision loss. In horses, glaucoma most commonly occurs secondary to equine recurrent uveitis (ERU).(1-3) Glaucoma has been estimated to affect 0.07% of the equine population in the United States;(4) however, the prevalence is likely underestimated due to intermittent and subtle clinical signs as well as lack of availability of instruments to measure IOP in equine practice. Clinical signs of glaucoma in the horse include episcleral injection, corneal edema, buphthalmia, corneal striae, pupil dilation, loss of vision and loss of pupil motility. Chronic and severe increases in intraocular pressure lead to destruction of retinal ganglion cells and their axons and cupping of the optic nerve. The normal mean IOP in horses measured by applanation tonometry ranges from 17 to 28.6 mmHg.(5-7)

Treatment of equine glaucoma involves management of any concurrent uveitis with systemic and/or topical non-steroidal anti-inflammatory drugs and topical administration of atropine as well as medical and/or surgical management of the elevated IOP. Medical glaucoma therapies evaluated and commonly used in horses include topical and systemic carbonic anhydrase inhibitors, topical beta-blockers, or topical combinations of a carbonic anhydrase inhibitor and beta-blocker.(8) Difficulties with the medical management of equine glaucoma include the relatively high cost of long-term medications and the limited ability of owners or trainers to treat affected eyes at the recommended frequency. Treatment with dorzolamide is most effective when administered every 8 hours.(9)

Cyclophotocoagulation, or laser destruction of the ciliary body, has been found to be an effective modality for lowering IOP by decreasing aqueous humor production in man,(10) dogs,(10-12) and horses.(2, 4, 13) The target of diode laser energy is the pigmented epithelium (PE) of the ciliary processes. Thermal coagulation of the PE, associated non-pigmented epithelium (NPE) and ciliary vasculature results in a decrease in the production of aqueous humor.(14-17) Two types of laser have been used for transscleral cyclophotocoagulation (TSCPC) in horses: Nd:YAG and semiconductor diode laser. Retrospective studies utilizing both types of laser have reported the efficacy of the procedure in horses for lowering IOP and retaining vision (Table 1).(2, 4, 13) The reported success rates in control of IOP and maintenance of vision range from 64-100% and 59-100%, respectively. These retrospective studies support the use of cyclophotocoagulation in equine glaucoma therapy, but interpretation of results is limited by the variation in laser type, procedure protocol, length and manner of follow-up and the retrospective nature of the collection of data including globe size.

A series of studies have investigated the histologic and clinical effects of semiconductor diode laser TSCPC on the ciliary body in normal equine eyes. First, an anatomic evaluation

of the equine pars plicata, in four quadrants of the equine globe was performed.(18) The pars plicata was observed to be very narrow in the nasal, dorsonasal and ventronasal quadrants and longer in the temporal, dorsotemporal and ventrotemporal quadrants. Based on this it was determined that the appropriate location for TSCPC to accurately target the equine ciliary processes was 4-6 mm posterior to the limbus in the dorsotemporal and ventrotemporal quadrants of the globe. Subsequently, the short-term histologic effects of TSCPC on the normal equine globe were evaluated at the recommended distances of 4 and 6 mm from the limbus at each quadrant and then a range of laser energy applications was evaluated.(19) This study determined that laser application 4 mm posterior to the limbus in the dorsotemporal and ventrotemporal quadrants at 1500 milliwatts (mW) for 1500 milliseconds (mSec) was most efficacious in coagulation of the ciliary body without undesirable destruction of adjacent tissues. Finally, the long-term histologic effects of diode laser on the ciliary body and the IOP was evaluated in normal equine eyes.(17) This study documented atrophy of ciliary processes as well as a significant and sustained reduction in IOP up to 24 weeks after TSCPC.

These previous studies of the anatomy of the normal equine ciliary body and the short and long-term effect of semiconductor diode laser TSCPC in the normal equine eye resulted in the recommendation to perform TSCPC 4 mm posterior to the limbus in the dorsotemporal and ventrotemporal quadrants using energy settings of 1500 milliwatts for 1500 milliseconds for a total energy of 2.25J/site.(17-19) To date, no study has evaluated the histologic effects when these recommendations are applied to TSCPC on a buphthalmic equine globe. The purpose of this study was to evaluate the effects of semiconductor diode TSCPC by previously described methods performed on buphthalmic equine globes to determine if the current recommended anatomic sites and energy levels are appropriate.

Materials and methods

Horses presented to The Ohio State University Veterinary Medical Center for enucleation of blind, buphthalmic globes were candidates for inclusion in the study. A complete ophthalmic examination including IOP by applanation tonometry, slitlamp biomicroscopy and indirect ophthalmoscopy were performed. The diagnosis of chronic glaucoma was made based on one or more of the following criteria: buphthalmia, corneal stria, IOP > 25 mmHg. Blindness was reported by the owner and was confirmed during examination based on a lack of menace and dazzle responses and abnormal pupillary light reflexes originating from the affected eye. The study was approved by The Ohio State University Veterinary Teaching Hospital Clinical Research/Teaching Advisory Committee, and informed consent for participation in the study was obtained from each horse's owner.

Under general anesthesia, immediately prior to enucleation, the globes were measured and TSCPC was performed. To measure axial globe length from the corneal epithelium to the posterior limit of the sclera, a 10 MHz ultrasound probe was applied to the axial cornea with an excess of sterile coupling gel used as a stand-off medium between the probe and corneal surface. B-mode ultrasonography with a machine-extrapolated A-mode tracing was used to obtain the measurements that were recorded in 11 of 12 cases. Axiality of the scan was ensured by a steep sharp retinal spike on the A-scan and visibility of aligned ultrasonic B-

mode echos from the anterior and posterior lens capsule and the center of the extraocular muscle cone. The axial globe measurements were recorded from the A-mode spike that coincided with the posterior limit of the sclera. In the last 5 cases, a Jameson caliper was also used to measure the axial globe length immediately following enucleation in order to compare measurements between the two techniques and assess the accuracy of ultrasonographic measurements performed *in situ*. Additionally, a Jameson caliper was used to measure the horizontal and vertical corneal diameter in all eyes.

In Part 1 of the study, TSCPC was performed on 9 globes at sixty sites 4 mm posterior to the limbus in the dorsotemporal and ventrotemporal quadrants, avoiding the 3:00 and 9:00 positions (Fig. 1), at the energy setting of 1500 milliwatts for 1500 milliseconds per site for a total energy of 2.25J/site. These settings were chosen based on the previously published recommendations.⁽¹⁹⁾

In Part 2, two additional buphthalmic globes had TSCPC performed as described above with the exception of the location of laser application. The new sites of laser site application were selected based on the histopathologic findings and histologic ciliary body measurements of the original 9 globes (Fig. 2). In both of these globes 15 spots were lasered at each of the following distances posterior to the limbus (Fig. 3): 8 mm in the dorsal quadrant from 12:00 for 1.5 clock hours, 6 mm in the dorsotemporal quadrant from approximately 10:30 for 1.5 clock hours, 5 mm in the ventrotemporal quadrant from approximately 9:00 for 1.5 clock hours and 5 mm in the ventral quadrant from approximately 8:30-6:00. A total of sixty sites were treated in each globe.

Case information that was collected was as follows: Age, breed, sex, historical, clinical and/or histologic evidence of ERU, duration of glaucoma, IOP at the time of presentation, prior medical treatment, axial globe length (mm) derived by B-scan and software derived A-scan ultrasonography, central horizontal corneal diameter (mm), central vertical corneal diameter (mm), axial globe length (mm) acquired by Jameson caliper measurement post-enucleation.

Enucleated globes were fixed in 10% formalin for a minimum of 24 hours. The globes were sectioned sagittally over the temporal aspect of the globe into two blocks to evaluate the dorsotemporal and ventrotemporal ciliary body. Histologic sections were taken at 1 mm intervals obtaining 5 histologic sections per block and 10 sections per eye (Fig. 1). The sections were stained with hematoxylin and eosin and examined by light microscopy by one examiner (AGM) for histopathologic changes.

For histologic review, the following regions of the uvea were examined and lesions attributable to laser application were recorded: Iris, anterior pars plicata, posterior pars plicata, pars plana and choroid. For each area of the uvea affected, presence or absence of the following changes were noted: Scleral coagulation, base of iris/ciliary process coagulation; tip of iris/ciliary process coagulation, pigment dispersion, separation/disruption of the PE/NPE, vascular congestion, inflammatory cells, disruption of architecture, retinal detachment. Additionally, coagulation of the pectinate ligament and trabecular meshwork and the underlying sclera were recorded.

After histologic review of all sections in Part 1, a representative slide from each tissue block for each eye was digitally imaged and the distance from the external limbus to the anterior margin of the pars plicata and from the external limbus to the anterior margin of the pars plana was determined. The range and mean of the measurements for all 9 globes was calculated and this was used to estimate the appropriate location for TSCPC application to achieve lesions only in the pars plicata.

Results

All twelve globes had one or more histologic findings compatible with chronic glaucoma including breaks in Descemet's membrane, recession and posterior bowing of the iris base, collapse of the iridocorneal angle, elongation and/or atrophy of the ciliary body, inner retinal degeneration, axonal degeneration in the optic nerve and optic nerve cupping.⁽¹⁾ One eye in Part 1 was excluded from the study due to lack of histologic lesions compatible with photocoagulation leaving 9 globes in Part 1 and 2 globes in Part 2.

The age of the eleven horses included in the study ranged from 6 to 26 years with a mean of 19.7 years and a median of 21 years. Breeds represented included 4 Quarter horses, 3 Appaloosas, 2 Thoroughbreds, 1 Haflinger and 1 Oldenburg. Seven globes were right eyes, including both globes in Part 2, and 4 globes were left eyes. Six horses were mares and 5 were gelded males. Equine recurrent uveitis (ERU) had been diagnosed previously in 6 of 11 eyes and the owner was unaware of any ophthalmic problems prior to the onset of the glaucoma in the remaining 5 eyes. Duration of ERU ranged from 6 months to 4 years with a mean of 2.6 years. Duration of glaucoma according the owner's history and prior clinical examinations ranged from 2 to 120 weeks (mean of 29 weeks). Treatment for glaucoma with dorzolamide +/- timolol had been initiated prior to presentation for enucleation in 9 of 11 eyes.

The IOP at the time of presentation for enucleation ranged from 9 to 64 mmHg (mean = 36.1 mmHg) and exceeded 25 mmHg in 8 of 11 globes. Vision loss was confirmed in 10 eyes by lack of menace and dazzle responses and absence of pupillary light reflexes originating from the affected globe. One eye in Part 2 of the study had markedly reduced menace and dazzle responses and pupillary light reflexes. The owner presented this horse for enucleation due to intractable discomfort during glaucoma episodes. Clinical findings of corneal edema and buphthalmia were noted in all 11 globes and Haab's corneal striae were recorded for 8 of 11 globes.

The axial globe length by ultrasonographic measurement was recorded for 10 of 11 globes and ranged from 40-52.5 mm (mean 46.3 mm). The axial globe length was measured by Jameson caliper in the last 5 of 11 globes admitted to the study and ranged from 42-50 mm (mean 46.5 mm). For these five eyes, the ultrasonographic versus the caliper measurements respectively were as follows: 45 mm vs. 48 mm; 41.7mm vs. 42 mm; 40 mm vs. 44 mm; 46.7 mm vs 50 mm; 47.1 mm vs. 48.5 mm. The corneal diameter ranged from 30-38 mm horizontally (mean 34.5 mm) and 22.5-29 mm vertically (mean 25.9 mm).

Part 1 Histologic Findings

When using the previously recommended location and laser settings for TSCPC of the equine eye in buphthalmic globes, common histologic lesions included coagulation of the sclera; coagulation of the stroma of the pars plicata (Fig. 4) and iris base (Fig. 5); uveal pigment dispersion, streaming and consolidation (Fig. 4); coagulation of ciliary processes; coagulation and disruption of the ciliary PE and NPE (Fig. 4); and uveal vascular congestion (Fig. 6). In all of the globes examined, substantial coagulation of the iris base and anterior ciliary processes was noted (Fig. 5). In 8 of 9 cases, coagulation of the pectinate ligaments was also observed (Fig. 7a,b). Histologic changes in the ciliary processes tended to affect the base more substantially than the tips of the processes and complete coagulation of the stroma including blood vessels was observed (Fig. 6). Histologic effects of the laser on the cornea, posterior pars plicata, pars plana or retina were not found in any case.

All histologic measurements for the location and length of the pars plicata are found in Table 2. The histologic measurements from the dorsal corneal limbus to the anterior pars plicata were longer than those from the ventral corneal limbus to anterior pars plicata (Fig. 8a). The measurements from the sections from the tissue block adjacent to the 12:00-6:00 meridian (eg. the more median section; Fig. 8a) were longer than those from the more temporal block (Fig. 8b). The range of the distance from the dorsal limbus to the anterior pars plicata was 4.1-8.7 mm while the mean distance for the more median sections was 6.1 mm and for the more temporal sections was 5.0 mm. From the histologic measurements of the location and length of the pars plicata, the sites in Figure 3 were determined to be appropriate for TSCPC in a buphthalmic equine globe.

In order to determine if the location of the pars plicata correlated with globe size, for each globe, the distance from the external limbus to the posterior aspect of the pars plicata was plotted against the ultrasonographic measurement of axial globe length (Fig. 9), against the horizontal corneal diameter (Fig. 10) and against the vertical corneal diameter (data not shown). A best-fit line on each graph indicated roughly linear correlation between axial globe length or horizontal corneal diameter and the location of the pars plicata, but not between vertical corneal diameter and location of the pars plicata.

Part 2 Histologic Findings

For both globes in Part 2, histologic laser effects similar to those in Part 1 were observed. However, coagulation and associated vascular and PE and NPE changes were confined to the pars plicata in a location posterior to the iris base in all histologic sections (Fig. 11a,b). In the dorsal quadrant, lesions were found in the robust ciliary processes immediately behind, but not involving the iris base. In the ventral and temporal quadrants, histologic lesions were found in a location posterior to the first ciliary process after the iris base in all histologic sections. No histologic lesions were observed in the cornea, pectinate ligament, iris base, trabecular meshwork, the ciliary processes that extended from the posterior iris base, the pars plana, choroid or retina.

Discussion

Buphthalmia in this study was confirmed by measurements of axial globe length and corneal diameter. The previously reported mean axial globe length by B-mode ultrasonic measurement of extirpated normal equine globes was 39.4 mm (34.6-45 mm) and by caliper was 40 mm (34-47 mm).(20) Another recent study reported similar ultrasonographic measurements of normal axial globe length with a mean of 40.04 mm (37.4-43.7 mm).(21) Reported manual measurements by caliper are always slightly longer than ultrasonic measurements and likely more accurate; however, good statistical correlation has been found between axial globe measurements taken *in-situ* ultrasonographically and in enucleated globes by Jameson caliper.(21) Comparisons between the two methods of measurement in our study were similar in that the mean axial length of buphthalmic globes determined by ultrasound and caliper measurement was 44.1 mm and 46.5 mm, respectively. When considering the results of these studies, it is highly likely that B-mode ultrasonography will provide accurate axial globe measurements in clinically buphthalmic equine globes. When compared with the normal equine globe, this study found a 17.5% and 10.3% increase in axial globe length by ultrasound and caliper, respectively.

The mean horizontal and vertical corneal diameter of the buphthalmic globes in this study was 34.5 mm and 24.9 mm respectively. These measurements are larger than the reported normal equine horizontal corneal diameter of 30.24 +/- 1.53 mm and central vertical diameter of 24.69 +/- 1.52 mm.(18) When compared with the normal equine globe, this study found a 21% increase in the horizontal corneal diameter and a 0.85% increase in the vertical corneal diameter in the buphthalmic globe.

The morphologic effects of globe stretching due to glaucoma reported in the horse include posterior bowing and recession of the iris base and iridocorneal angle relative to the limbus. (1) When the laser probe was applied to buphthalmic globes at 4 mm posterior to the limbus, the iris base and iridocorneal angle were affected due to these morphologic changes. Although statistical analysis was not performed due to the small number of data points, a roughly linear correlation was found between either axial globe length or horizontal corneal diameter and the distance from the dorsal external limbus to the pars plicata. This implies that the measurable parameters that confirm stretching of the globe can be used to estimate the appropriate increase in distance from the external limbus for laser probe application. In other words, in a more buphthalmic globe, the laser probe should be applied at a distance more posterior to the limbus to affect the pars plicata than in a normally-sized or mildly buphthalmic globe.

The location and length of the ciliary body in horses varies by region of the globe as is depicted in Figure 2. In a study of normal ciliary body anatomy, the internal length of the pars plicata ranged from 5.33 +/- 0.49 mm to 10.67 +/- 1.15 mm.(18) Dorsally near 12:00, the pars plicata is located more posteriorly to the limbus and is longer in length than ventrally near 6:00. Because the corneal limbus in the horse is horizontally flattened while the approximate shape of the pars plicata is circular, the distance from the limbus to the pars plicata and pars plana is shorter in the temporal quadrant even though the pars plicata is longest at that location. Therefore, based on normal anatomic studies and the histologic

findings and measurements in our study, the recommended sites for laser application must also vary according to the location on the globe with the longest distance posterior to the limbus located dorsally and shorter distances located ventrally and temporally.

Histologic effects of the TSCPC procedure in buphthalmic equine globes were similar to those in previous studies in man, dogs, rabbits and horses.(14, 16, 19, 22, 23) Coagulation of uveal stromal, vascular and epithelial tissue occurred as expected in all cases. Signs of excessive damage induced by the laser, such as extensive disruption of uveal tissue architecture or excessive hemorrhage, were not seen in any case. This indicates that the previously recommended energy setting and duration of laser application per spot is appropriate in buphthalmic equine globes.(19) The results of Part 1 of this study, however, clearly demonstrated that the previously published recommended sites for TSCPC based on studies in anatomically normal equine globes are located too far anteriorly to target the pars plicata in even mildly buphthalmic equine globes. Although the anterior ciliary body including the large ciliary processes that extend from the posterior base of the iris were affected, so was the iris base and pectinate ligament. Coagulation of the iris base is undesirable as it is likely to cause unnecessary uveitis, discomfort and potentially dyscoria due to damage to the iris stroma, musculature and nerves. Coagulation of the underlying trabecular meshwork and the pectinate ligament exacerbates damage to the iridocorneal angle, further impairing aqueous outflow. Furthermore, the application of the laser probe 4 mm posterior to the limbus likely lowers the clinical efficacy of the procedure in that it does not result in maximal effect on the target tissue, which is the pars plicata. It is possible that this is at least part of the reason for the suboptimal long-term efficacy of TSCPC in horses or for complete failure of TSCPC to reduce the IOP in some severely buphthalmic equine eyes. Interestingly, when considering the results of all three retrospective studies of TSCPC in horses, the highest success rates in IOP control and vision retention are reported in the study where cases were lasered 5-6 mm posterior to the limbus.(4) This is in contrast to the lowest rate of IOP control and vision retention in the study where cases were lasered 4 mm posterior to the limbus.(2)

In Part 2 of this study where the TSCPC locations were moved more posteriorly, all histologic changes were confined to the pars plicata. The glaucoma probe for TSCPC application can be safely aimed at the more posterior aspects of the proposed ranges in the dorsal quadrant, especially in more buphthalmic globes since histologic lesions in the dorsal quadrant involved mainly the ciliary processes immediately behind the iris base. For the ventral quadrant, the more anterior aspects of the proposed ranges can be targeted, especially in less buphthalmic globes, since it was observed in one Part 2 globe that the more robust ciliary processes of the anterior pars plicata were spared and the lesions primarily affected the middle aspect of the ciliary body. Both globes were lasered at the most posterior aspects of the recommended ranges to confirm that within those ranges, the pars plana or retina would not be affected. The biometric axial globe length and corneal diameter measurements of globes in Part 2 were comparable to those in Part 1. The number of globes included in this study was not sufficient to perform regression analysis to determine more definitive sites for laser application based on axial globe or horizontal corneal diameter measurements. However, based on the findings of this study, the newly proposed sites for laser application should be safe to apply to globes that range from mildly to severely enlarged and the site of

laser application could be staggered within the proposed ranges to achieve a maximal effect on the pars plicata.

Although edema and disruption of the ciliary PE and NPE was observed at the tip of some ciliary processes, histologic changes in the pars plicata tended to affect the base of the ciliary processes more substantially than the tips, raising the question as to whether either an increase in laser energy or a longer duration of laser application per site may be necessary for more complete destruction of entire ciliary processes. However, since histologic evaluation was performed immediately after the laser procedure was performed, it is unknown whether destruction of the base of the ciliary process where blood supply enters the process would subsequently result in complete atrophy of the process. Multiple studies suggest that this is likely the case. Coagulation of the stroma and vasculature of the ciliary body was noted histologically in this study as well as in studies of laser effects on the ciliary body in rabbits.(24, 25) In dogs, ciliary body necrosis and hemorrhage was documented at 7 days and ciliary body atrophy and fibrosis at 28 days after TSCPC.(15) Work in horses documented long-term atrophy of the ciliary body as well as a sustained decrease in IOP in normal horses after diode laser TSCPC at the same laser settings used in the current study. (17) It would be expected that the energy and duration of laser application should be the same in a buphthalmic globe where the fibrous tunic has stretched and the sclera is thinner because the 810 nm wavelength of the diode laser is absorbed by the melanin-containing uvea rather than the sclera.

Several limitations of this study must be acknowledged. First, the number of globes evaluated in the study was small due to lack of availability of candidates fitting the inclusion criteria over the 12 years of enrolling cases. However, the histologic findings were consistent in all of the globes thereby adding strength to the data and conclusions. Second, the histologic measurements performed are not likely highly accurate. Tissue shrinkage from formalin and distortion of the round shape of the globe within the wax blocks and on the glass slides could have altered the measurements; it was not usually possible to use a single straight line of measurement. Rather, in some cases, two lines of measurement were added together. Third, in some globes with sparse periocular pigment, it was sometimes difficult to definitively identify the exact location of the external limbus. Finally, the same relative histologic section could not be measured for each globe. The goal was to measure one section that was closest to the median plane (12:00-6:00) and the most median section from the temporal block. However, sections that had higher quality staining and ability to visualize morphologic landmarks were selected for scanning and measurement in some cases. The most temporal sections were posterior to the limbus such that limbal to pars plicata and pars plana measurements could not be obtained. Therefore, the histologic measurements on the scanned slides were merely a way to estimate an appropriate distance from the limbus for laser application.

The anticipated and previously described effects of diode laser on the ciliary body were observed in buphthalmic equine globes. However, the histologic findings of coagulation and vascular congestion of the iris base and/or pectinate ligament in all buphthalmic globes indicate that the previously recommended laser site of 4 mm posterior to the limbus is too anterior for equine globes that are enlarged from glaucoma. To adequately target the ciliary

processes during TSCPC in buphthalmic equine globes, the laser probe should be aimed at the following distances posterior to the limbus: 6-8 mm dorsally, 5-6 mm dorsotemporally, 4-5 mm ventrotemporally, and 4-5 mm ventrally. Further investigation of the clinical efficacy of diode laser TSCPC on buphthalmic equine globes at the newly proposed sites is warranted to confirm the appropriate location as well as energy and duration settings for laser application.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

Acknowledgments

The authors would like to thank the following people who provided support for this project: Alan Flechtner for preparation of histologic specimens; Dr. Michelle Willis for her intellectual contribution to this project; Drs. Raymond Morreale, Vanessa Kuonen, Sarah Stone, David Haeussler and Elizabeth Lutz for their assistance in recruiting and processing cases; Tim Vojt for biomedical illustrations; The Ohio State University Comparative Pathology & Mouse Phenotyping Shared Resource (CPMPSR) supported in part by NCI grant P30 CA016058.

References

1. Curto EM, Gemensky-Metzler AJ, Chandler HL, Wilkie DA. Equine glaucoma: a histopathologic retrospective study (1999-2012). *Vet Ophthalmol*. 2013
2. Annear MJ, Wilkie DA, Gemensky-Metzler AJ. Semiconductor diode laser transscleral cyclophotocoagulation for the treatment of glaucoma in horses: a retrospective study of 42 eyes. *Vet Ophthalmol*. 2010; 13(3):204–9. [PubMed: 20500721]
3. Cullen CL, Grahn BH. Equine glaucoma: a retrospective study of 13 cases presented at the Western College of Veterinary Medicine from 1992 to 1999. *Can Vet J*. 2000; 41(6):470–80. [PubMed: 10857031]
4. Miller TR, Brooks DE, Gelatt KN, King TC, Smith PJ, Sapienza JS, et al. Equine glaucoma: clinical findings and response to treatment in 14 horses. *Vet & Comp Ophthalmol*. 1995; 5(3):170–82.
5. van der Woerd A, Gilger B, Wilkie DA, Strauch SM. Effect of auriculopalpebral nerve block and intravenous administration of xylazine on intraocular pressure and corneal thickness in horses. *Am J Vet Res*. 1995; 56(2):155–8. [PubMed: 7717576]
6. Miller PE, Pickett JP, Majors LJ. Evaluation of two applanation tonometers in horses. *Am J Vet Res*. 1990; 51(6):935–7. [PubMed: 2368953]
7. Dziezyc J, Millichamp NJ, Smith WB. Comparison of applanatin tonometers in dogs and horses. *JAVMA*. 1992; 201(3):430–3. [PubMed: 1506245]
8. Willis AM, Robbin TE, Hoshaw-Woodard S, Wilkie DA, Schmall ML. Effect of topical administration of 2% dorzolamide hydrochloride or 2% dorzolamide hydrochloride-0.5% timolol maleate on intraocular pressure in clinically normal horses. *Am J Vet Res*. 2001; 62(5):709–13. [PubMed: 11341390]
9. Gelatt JK, MacKay EO. Changes in intraocular pressure associated with topical dorzolamide and oral methazolamide in glaucomatous dogs. *Vet Ophtho*. 2001; 4:61–7.
10. Ghosh S, Manvikar S, Ray-Chaudhuri N, Birch M. Efficacy of transscleral diode laser cyclophotocoagulation in patients with good visual acuity. *Eur J Ophthalmol*. 2013:0.
11. Cook CS, Davidson MG, Brinkman M, Priehs D, Abrams KL, Nasisse MP. Diode laser transscleral cyclophotocoagulation for the treatment of glaucoma in dogs: results of six and twelve month follow-up. *Vet & Comp Ophtho*. 1997; 7(3):148–54.
12. Hardman C, Stanley RG. Diode laser transscleral cyclophotocoagulation for the treatment of primary glaucoma in 18 dogs: a retrospective study. *Vet Ophthalmol*. 2001; 4(3):209–15. [PubMed: 11722786]

13. Whigham HM, Brooks DE, Andrew SE, Gelatt KN, Strubbe DT, Biros DJ. Treatment of equine glaucoma by transscleral neodymium:yttrium aluminum garnet laser cyclophotocoagulation: a retrospective study of 23 eyes of 16 horses. *Vet Ophthalmol.* 1999; 2(4):243–50. [PubMed: 11397272]
14. Blasini M, Simmons R, Shields MB. Early tissue response to transscleral neodymium: YAG cyclophotocoagulation. *Invest Ophthalmol Vis Sci.* 1990; 31(6):1114–8. [PubMed: 2354913]
15. Nasisse MP, Davidson MG, MacLachlan NJ, Corbett W, Tate LP, Newman HC, et al. Neodymium:yttrium, aluminum, and garnet laser energy delivered transsclerally to the ciliary body of dogs. *Am J Vet Res.* 1988; 49(11):1972–8. [PubMed: 3247921]
16. Nasisse MP, McGahan MC, Shields MB, Echelman D, Fleisher LN. Inflammatory effects of continuous-wave neodymium: yttrium aluminum garnet laser cyclophotocoagulation. *Invest Ophthalmol Vis Sci.* 1992; 33(7):2216–23. [PubMed: 1318869]
17. Cavens VJ, Gemensky-Metzler AJ, Wilkie DA, Weisbrode SE, Lehman AM. The long-term effects of semiconductor diode laser transscleral cyclophotocoagulation on the normal equine eye and intraocular pressure(a). *Vet Ophthalmol.* 2012; 15(6):369–75. [PubMed: 22288723]
18. Miller TL, Willis AM, Wilkie DA, Hoshaw-Woodard S, Stanley JR. Description of ciliary body anatomy and identification of sites for transscleral cyclophotocoagulation in the equine eye. *Vet Ophthalmol.* 2001; 4(3):183–90. [PubMed: 11722782]
19. Morreale RJ, Wilkie DA, Gemensky-Metzler AJ, Weisbrode SE, Willis MA. Histologic effect of semiconductor diode laser transscleral cyclophotocoagulation on the normal equine eye. *Vet Ophthalmol.* 2007; 10(2):84–92. [PubMed: 17324163]
20. Rogers M, Cartee RE, Miller W, Ibrahim AK. Evaluation of the extirpated equine eye using B-mode ultrasonography. *Vet Radiol.* 1986; 27(1):24–9.
21. Grinninger P, Skalicky M, Nell B. Evaluation of healthy equine eyes by use of retinoscopy, keratometry, and ultrasonographic biometry. *Am J Vet Res.* 2010; 71(6):677–81. [PubMed: 20513184]
22. Nasisse MP, Davidson MG, English RV, Jamieson V, Harling DE, Tate LP. Treatment of glaucoma by use of transscleral neodymium:yttrium aluminum garnet laser cyclocoagulation in dogs. *J Am Vet Med Assoc.* 1990; 197(3):350–4. [PubMed: 2391270]
23. Nadelstein B, Wilcock BP, Cook CS, Davidson MG. Clinical and histopathologic effects of diode laser transscleral cyclophotocoagulation in the normal canine eye. *Vet & Comp Ophtho.* 1997; 7(3):155–62.
24. Schlote T, Beck J, Rohrbach JM, Funk RH. Alteration of the vascular supply in the rabbit ciliary body by transscleral diode laser cyclophotocoagulation. *Graefe's archive for clinical and experimental ophthalmology = Albrecht von Graefes Archiv fur klinische und experimentelle Ophthalmologie.* 2001; 239(1):53–8.
25. Brancato R, Leoni G, Trabucchi G, Cappellini A. Histopathology of continuous wave neodymium: yttrium aluminum garnet and diode laser contact transscleral lesions in rabbit ciliary body. A comparative study. *Invest Ophthalmol Vis Sci.* 1991; 32(5):1586–92. [PubMed: 2016140]

Figure 1.

Diagram of an equine globe. The red dots demonstrate the location of sites of laser application 4 mm posterior to the limbus. The vertical black lines indicate the histologic blocks examined (block 1 is more median and block 2 is more temporal). Five histologic sections were processed from each block. D=dorsal, T=temporal, V=ventral, N=nasal. Image from Cavens et. al. The long-term effects of semiconductor diode laser transscleral cyclophotocoagulation on the normal equine eye and intraocular pressure. *Vet Ophthalmol* 2012;15(6):369-375.

Figure 2.

Diagram of the internal anatomy of an equine globe. The purple indicates the ciliary body. The ridged purple tissue indicates the pars plicata. The non-textured purple tissue indicates the pars plana. The oval white dotted line indicates the location of the external limbus of the cornea. The lens is depicted in the center. T=temporal, N=nasal. The green lines and associated number values indicate the estimated appropriate distances from the external limbus for laser probe application during TSCPC in a buphthalmic equine globe.

Figure 3.

Diagram of an equine globe. The red dots demonstrate the location of sites of laser application in a buphthalmic globe. The labels in each quadrant (clockwise from the “D”: dorsal, dorsotemporal, ventrottemporal and ventral) identify the recommended distance posterior to the limbus for laser application. D=dorsal, T=temporal, V=ventral, N=nasal, mm=millimeters.

Figure 4.

Photomicrograph of a diode laser induced lesion of the pars plicata region of the ciliary body. Photocoagulation of the sclera and stroma as well as the ciliary processes is evidenced by basophilic color change, loss of collagen fibrillar architecture and pigment dispersion and streaming. Disruption of the ciliary pigmented and non-pigmented epithium has occurred. Hematoxylin and eosin, 200X.

Figure 5.

Photomicrograph of a diode laser induced lesion of the iris base, pectinate ligaments and iridocorneal angle. Photocoagulation of the stroma is evidenced by basophilic color change, loss of collagen fibrillar architecture and pigment dispersion and streaming. The termination of Descemet's membrane inserting on the pectinate ligaments can be seen on the bottom left. Hematoxylin and eosin, 40X.

Figure 6.

Photomicrograph of a diode laser induced lesion of the pars plicata region of the ciliary body. Photocoagulation of the sclera and stroma as well as the ciliary processes is evidenced by basophilic color change, loss of collagen fibrillar architecture and pigment streaming. Note the disruption of the non-pigmented epithelium and complete coagulation of the stromal blood vessels and the adjacent vascular congestion. Hematoxylin and eosin, 40X.

Figure 7.

a-b. Photomicrographs of the pectinate ligaments of the superior and inferior iridocorneal angle in a 21-year-old Quarter horse mare. a) Photocoagulation in the superior pectinate ligaments is evidenced by basophilic color change, loss of collagen fibrillar architecture and pigment dispersion and streaming. b) The pectinate ligaments in the inferior iridocorneal angle have not been affected by laser application. Hematoxylin and eosin, 200X.

Figure 8.

a,b. Aperio scanned photomicrographs of the globe of a 20-year-old Haflinger gelding. Measurements were taken from the histologic external limbus to the most anterior aspect of the pars plicata in representative sections from the more median (a) and the more temporal blocks (b). A) In this median section, the corpora nigra at the edge of the dorsal iris leaflet and the ventral location of the optic nerve provide orientation. Dorsally the distance from limbus to pars plicata (7.155 mm) is longer than the same measurement ventrally (6.486 mm); B) In this more temporal section, the limbal to pars plicata measurement (4.005 mm) is shorter than the measurements in the median section.

Figure 9.

Distance from the dorsal limbus to the posterior margin of the pars plicata versus axial globe length by ultrasonographic measurement. A best fit line indicates roughly linear correlation between globe size and length of the ciliary body in a buphthalmic equine globe.

Figure 10.

Distance from the dorsal limbus to the posterior margin of the pars plicata versus horizontal corneal diameter. A best fit line indicates roughly linear correlation between horizontal corneal diameter and length of the ciliary body in a buphthalmic equine globe.

Figure 11.

a,b. a) Photomicrograph of the unaffected inferior pectinate ligaments, iris base, and iridocorneal angle at the bottom left in a Phase 2 globe lasered at the more posterior sites. Photocoagulation of the adjacent ciliary processes on the upper right is evidenced by basophilic color change, loss of collagen fibrillar architecture and pigment dispersion and streaming. Hematoxylin and eosin, 40X. b) Photomicrograph of the superior pars plicata immediately posterior to the large vessels of the iris base. Photocoagulation of the sclera and ciliary body stroma and processes is evidenced by basophilic color change, loss of collagen fibrillar architecture and pigment dispersion and streaming. Hematoxylin and eosin, 200X.

Table 1
Comparison of retrospective studies evaluating the efficacy of cyclophotocoagulation in horses with glaucoma.

Study (Laser type)	Number of eyes	Distance of laser application from limbus	Percentage of eyes with IOP control	Percentage of eyes sighted	Length of follow-up
TR Miller et al. 1995 (Nd:Yag)	8	5-6 mm	100%	100%	9-24 months
Whigham et al 1999 (Nd:Yag)	23	5 mm	70%	60%	5 months
Annear et al 2010 (Diode)	42	4 mm	64%	100%	5-17 months
			-----	59%	49 months (telephone)

Table 2

Histologically measured distance from dorsal corneal limbus to anterior pars plicata and ventral corneal limbus to anterior pars plicata.

Dorsal limbus to pars plicata	
Range	4.1-8.7 mm
Mean 12:00 meridian	6.1 mm
Mean temporal	5.0 mm
Ventral limbus to pars plicata	
Range	3.1-6.5 mm
Mean 6:00 meridian	5.2 mm
Mean temporal	4.3 mm
Dorsal limbus to pars plana	
Range	9.1-13.8 mm
Mean 12:00 meridian	11.4 mm
Ventral limbus to pars plana	
Range	6.7-13.0 mm
Mean 6:00 meridian	10.1 mm