ผลการเปรียบเทียบการตั้งค่าเลเซอร์ที่แตกต่างกัน 3 วิธีการในการใช้เลเซอร์ชนิดไดโอดผ่านเปลือกหุ้มลูกตาเพื่อรักษาต้อหินเรื้อรังในสุนัข

นางสาวชมพูนุท เพิ่มคำ

วิทยานิพนธ์นี้เป็นส่วนหนึ่งของการศึกษาตามหลักสูตรปริญญาวิทยาศาสตรมหาบัณฑิต
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ปีการศึกษา 2558

A COMPARISON OF THREE DIFFERENT SETTING PROTOCOLS OF TRANSSCLERAL DIODE LASER CYCLOPHOTOCOAGULATION IN CHRONIC CANINE GLAUCOMA

Miss Chompunut Permkam

A Thesis Submitted in Partial Fulfillment of the Requirements
for the Degree of Master of Science Program in Veterinary Surgery
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ชมพูนุท เพิ่มคำ : ผลการเปรียบเทียบการตั้งค่าเลเซอร์ที่แตกต่างกัน 3 วิธีการในการใช้เลเซอร์ชนิดไดโอดผ่านเปลือกหุ้มลูกตาเพื่อรักษาต้อหินเรื้อรังในสุนัข (A COMPARISON OF THREE DIFFERENT SETTING PROTOCOLS OF TRANSSCLERAL DIODE LASER CYCLOPHOTOCOAGULATION IN CHRONIC CANINE GLAUCOMA) อ.ที่ปรึกษาวิทยานิพนธ์หลัก: ผศ. สพ.ญ. ดร.นลินี ตันติวนิช, 76 หน้า.

การใช้เลเซอร์ชนิดไดโอดผ่านเปลือกหุ้มลูกตาเพื่อทำลายแขนงของซิลิอารี ทำให้เกิดการตายของเนื้อเยื่อแบบจับตัวเป็นก้อน วิธีการนี้มีจุดประสงค์เพื่อลดความดันในลูกตา โดยการใช้หลักการที่แสงเปลี่ยนเป็นความร้อน ทำให้เยื่อบุผิวของซิลิอารีตายแบบจับตัวเป็นก้อน ส่งผลต่อการสร้างของเหลวในลูกตาที่ลดน้อยลง จึงสามารถควบคุมความดันในภาวะต้อหินได้ การศึกษานี้เป็นการศึกษาเปรียบเทียบผลทางคลินิก และการเปลี่ยนแปลงทางพยาธิวิทยาของซิลิอารีบอดี เมื่อใช้วิธีการตั้งค่าเลเซอร์ที่แตกต่างกัน 3 วิธี ในการรักษาต้อหินเรื้อรังในสุนัข โดยวิธีการแรก ตั้งค่าพลังงาน 1500 มิลลิวัตต์ในระยะเวลา 1500 มิลลิวินาที จำนวนการยิงเลเซอร์ 40 ตำแหน่ง เป็นวงรอบ 270 องศาของลูกตา ห่างจากลิมบัส 3, 4 และ 5 มิลลิเมตร ส่วนวิธีการที่ 2 ตั้งค่าพลังงาน ระยะเวลา และระยะทางที่เหมือนกับในวิธีการแรก แต่จำนวนการยิงเลเซอร์ 80 ตำแหน่ง และวิธีการที่ 3 ตั้งค่าพลังงาน 1000 มิลลิวัตต์ในระยะเวลา 2500 มิลลิวินาที จำนวนการยิงเลเซอร์ 80 ตำแหน่ง เป็นวงรอบ 360 องศาของลูกตา ห่างจากลิมบัส 3 และ 4 มิลลิเมตร ผลการศึกษาพบว่าแต่ละวิธีการมีอัตราความสำเร็จในการควบคุมความดันให้ต่ำกว่า 21 มิลลิเมตรปรอท เท่ากับ 60%, 90% และ 100% โดยวิธีการที่ 1, 2 และ3ตามลำดับ นอกจากนี้ยังพบว่าจำนวนตำแหน่งของการยิงเลเซอร์ และพลังงานของเลเซอร์รวมต่อลูกตา มีความสัมพันธ์อย่างมากต่อความสำเร็จในการลดความดันในลูกตา ซึ่งการลดลงของความดันในลูกตา, ปริมาณการใช้ยาหยอดตาเพื่อควบคุมความดัน และความยาวตามแนวแกนของลูกตาของแต่ละวิธีการเมื่อนำมาเปรียบเทียบพบว่ามีความใกล้เคียงกัน ภาวะแผลหลุมที่กระจกตาเป็นภาวะแทรกซ้อนที่พบได้มากที่สุดในการศึกษานี้ ในขณะที่การอักเสบในช่องหน้าตาเป็นภาวะแทรกซ้อนสำคัญที่ทำให้ไม่สามารถควบคุมความดันในลูกตา และนำไปสู่การผ่าตัดเพื่อเอาลูกตาออก ซึ่งพบได้ในวิธีการที่ 1 และ 2 การศึกษาทางพยาธิวิทยาพบการเปลี่ยนแปลงที่มีลักษณะใกล้เคียงกันในแต่ละวิธีการ คือพบการตายของเนื้อเยื่อแบบจับตัวเป็นก้อน และการแยกชั้นของเนื้อเยื่อซิลิอารีภายหลังจากได้รับความร้อนจากเลเซอร์ แต่ระดับของการเปลี่ยนแปลงมีความรุนแรงแตกต่างกัน ในขณะที่ส่วนของพาร์ส พลานา ไม่เกิดการเปลี่ยนแปลง

ภาควิชา ศัลยศาสตร์ ลายมือชื่อนิสิต .....

สาขาวิชา ศัลยศาสตร์ทางสัตวแพทย์ ลายมือชื่อ อ.ที่ปรึกษาหลัก .....

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5675302931 : MAJOR VETERINARY SURGERY
KEYWORDS: TRANSSCLERAL CYCLOPHOTOCOAGULATION / SEMICONDUCTOR DIODE LASER / DIFFERENT PROTOCOLS / CHRONIC / CANINE GLAUCOMA

CHOMPUNUT PERMKAM: A COMPARISON OF THREE DIFFERENT SETTING PROTOCOLS OF TRANSSCLERAL DIODE LASER CYCLOPHOTOCOAGULATION IN CHRONIC CANINE GLAUCOMA. ADVISOR: ASST. PROF. NALINEE TUNTIVANICH, D.V.M., Ph.D., DAiCVO, D.T.B.V.S., 76 pp.

Transscleral diode laser cyclophotocoagulation is the method to lower the intraocular pressure by causing coagulative necrosis of ciliary epithelium, resulting in a decrease of aqueous humor production. This study was to compare clinical outcome and pathological changes of ciliary body, using three different setting protocols in chronic canine glaucoma. Protocol I 1500:1500 (power:duration), 40 spots, 270 degrees around the globe at 3, 4 and 5 mm posterior to the limbus, Protocol II 1500:1500 (power:duration), 80 spots, at the area as described in protocol I, and Protocol III 1000:2500 (power:duration), 80 spots, 360 degrees circumferential at 3 and 4 mm behind the limbus. Rate of success in reducing intraocular pressure was 60%, 90% and 100% in protocols I, II and III, respectively. Number of laser spots and total energy delivery per eye were highly correlated to the success. Reduction of intraocular pressure, topical hypotensive medication application and axial globe length were relatively comparable among the three protocols. Ulcerative keratitis was the main complication in all protocols while aqueous flare was the major cause of uncontrollable intraocular pressure, leading to enucleation in protocols I and II. Coagulative necrosis and ciliary tissue separation were found in all protocols with different extent, while pars plana was intact.

Department: Veterinary Surgery Student's Signature .....
Field of Study: Veterinary Surgery Advisor's Signature .....
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List of Abbreviations

μm	=	micrometer
°C	=	degree Celsius
AGL	=	axial globe length
ALP	=	alkaline phosphatase
ALT	=	alanine transaminase
ANOVA	=	analysis of variance
BUN	=	blood urea nitrogen
CA	=	carbonic anhydrase
CI	=	confidence interval
ECP	=	endoscopic cyclophotocoagulation
G	=	gauge
G-probe	=	glaucoma-probe
H&E	=	Hematoxylin and Eosin
hr	=	hour
IOP	=	intraocular pressure
kg	=	kilogram
M	=	molar
mg	=	milligram
ml	=	milliliter
mm	=	millimeter

mmHg	=	millimeter of mercury
ms	=	millisecond
mW	=	milliwatt
n	=	number
Nd:YAG	=	Neodymium-doped : Yttrium Aluminium Garnet
nm	=	nanometer
No.	=	number
PAS	=	Periodic Acid Schiff
PIFM	=	pre-iridal fibrovascular membrane
r	=	correlation coefficient
R^2	=	coefficient of determination
SD	=	standard deviation
TSCP	=	transscleral cyclophotocoagulation

Chapter 1

Introduction

Importance and Rationale

Glaucoma is one of the most common ocular diseases leading to irreversible blindness in dogs worldwide. The disease is majorly characterized by elevated intraocular pressure (IOP) from an imbalance between production and outflow of aqueous humor. As a result, degenerative changes of the optic disc and retinal ganglion cells occur. Incidence of glaucoma has continuously increased and to be exponentially incremented in the future (Tham et al., 2014).

To maintain vision in glaucoma patients, Glaucoma Research Foundation has suggested 5 common tests for accurate diagnosis in humans; (1) measurement of IOP (2) examination of optic disc morphology (3) visual field test (4) gonioscopy and (5) measurement of central corneal thickness. With all tests together, not only misdiagnosis is avoided, prompt treatment and prediction of disease progression can be achieved.

The goal of glaucoma treatment is to preserve vision and to minimize ocular pain. To that purposes, decreasing IOP by reduction of aqueous humor and/or facilitation of aqueous drainage should be achieved soon enough. Medical therapy with topical hypotensive drugs is still first treatment of choice. New products have endlessly launched into the market with claims of better result, efficacy, prolonged effectiveness, more convenient compliance, more ocular surface comforting and less undesirable side effects (Denis, 2011). However, hypotensive medications when used alone were inadequate for controlling the disease and their cost is also high with a lifelong treatment (Ting et al., 2014). Alternative therapy has then become more promising for long-term treatment of canine glaucoma.

Nowadays, cyclophotocoagulation has gained more popularity as a treatment of glaucoma in human medicine. As compared to other therapy, it provides desirable IOP lowering effect in addition to less ocular surface complications (Cook C, 1997). Transscleral cyclophotocoagulation (TSCP) is a non-invasive procedure by which laser energy delivered through the sclera to pars plicata (Brancato et al., 1991). With laser being absorbed by pigments, photocoagulative necrosis occurs at non-pigmented ciliary epithelial cells. It results in destruction of aqueous humor producing cells, lowering aqueous production and accumulation, then reduction of IOP.

One of the most common lasers used in animals is semiconductor diode laser that emits light of a wavelength of 810 nm (Spiess, 2012). While diode laser TSCP provides high clinical success, it demonstrates clinical complications caused by photodisruption (Lin et al., 2006) as well as inadvertent collateral damage at the laser site (Schuman et al., 1990). Optimal diode laser TSCP protocol should be considered to gain the balance between high clinical success and less clinical complications. However, it is still controversial whether there is a decent TSCP protocol that fits all types of the disease in dogs.

While prevalence of canine glaucoma is increasing and glaucomatous eyes have ended up with enucleation, understanding of TSCP in veterinary practice is nevertheless limited. With the purpose of studying TSCP parameters that plays an important role in the success of TSCP, three different setting TSCP protocols were studied to access correlation of TSCP parameters. Structural alterations of diode laser target tissues and surrounding area were identified.

Research questions

Primary research questions

1. Are three different TSCP protocols effective in IOP reduction, decrease of hypotensive drug application and reduction of axial globe length?
2. Are TSCP-treated eyes undergone compromised pathological changes?

Secondary research questions

1. What are undesirable ophthalmic complications of each TSCP protocols?
2. Are total energy of laser delivery per eye statistically correlated to clinical outcomes of the study and to ophthalmic complications?

Objectives of the study

1. To investigate ophthalmic outcomes of chronic glaucomatous eyes following different TSCP protocols
2. To study the pathological changes of ciliary body of chronic glaucomatous eyes following different TSCP protocols.

Advantage of the study

Appropriate TSCP setting, not only succeeds in clinical treatment of glaucoma, but also creates more localized histological changes of target tissue while minimizing laser damage to non-target tissue. Correlation of various TSCP parameters is beneficial for a determination of effective protocol setting for individuals.

CHAPTER 2

Literature review

Canine glaucoma

Canine glaucoma is a group of ocular diseases that exhibits increased level of pressure within the eye. Elevation of the IOP is harmful to the preservation of vision. Due to the fact that increased IOP interferes axoplasmic flow in retinal ganglion cells, blindness is the result (Gelatt et al., 2008). Because mean age of people has nowadays increased, risk of developing glaucoma has therefore risen worldwide (Tham et al., 2014).

Prevalence model of glaucoma was studied in humans to estimate the number of glaucoma cases worldwide. It predicted that there would be more people suffering from glaucoma by an increase in number of old people and more availability of glaucoma treatments (Quigley and Broman, 2006). Martin in 1977 first reported the prevalence of the canine glaucoma as 0.5% in the North America. The prevalence of primary or breed-related glaucoma and secondary glaucoma in dogs has been gradually increasing. Hence, the combined prevalence of primary and secondary glaucoma in dogs during the past decade was 1.7% which was comparable to the report of 1-2% worldwide prevalence of glaucoma in humans (Gelatt et al., 2008). Similar to canine glaucoma epidemiology study in Switzerland from 1995-2009, dogs presented at the University of Zurich ophthalmology service were diagnosed with glaucoma 7.15% (428 from 5984 dogs) which could be divided into congenital glaucoma 1.17% (5 out of 428 dogs), primary glaucoma 28.74% (123 from 428 dogs), secondary glaucoma 50.70% (217 out of 428 dogs) and 19.39% (83 cases from 428 dogs) with unknown etiology (Strom et al., 2011).

Classification

Canine glaucoma can be classified into two types according to pathogenesis; primary and secondary. Primary glaucoma has no obvious association with another ocular or systemic disorder. It is typically bilateral and predisposed to some breeds, hence it is believed to have a genetic basis. Primary glaucoma is subdivided into two main characteristics. First is a malfunction of pectinate ligaments in the iridocorneal angle (primary closure angle glaucoma). Second is an accumulation of glycosaminoglycans in trabecular meshwork (open angled glaucoma). American cocker spaniel, Basset Hound, Samoyed and Siberian husky were reported with primary closure angle glaucoma (Grozdanic et al., 2010) while primary open angle glaucoma was documented in Beagle (Gelatt et al., 1981).

Secondary glaucoma occurs in association with other ocular or systemic disorders (Gelatt et al., 2008). It may be unilateral or bilateral or may not be inherited (Miller, 2008). Pathogenesis of secondary glaucoma can be defined into 3 mainly mechanisms altering aqueous humor dynamics; obstruction of the iridocorneal angle (inflammatory cells, hyphema, or neoplastic cells), pupillary block (anterior or posterior synechia), and ciliary body-vitreous-lens block (lens subluxation or luxation).

Figure 1 Picture demonstrated a dog with marked globe enlargement (buphthalmos) of left eye from chronic glaucoma.

Clinical signs

When IOP dramatically increases, dogs acutely develop ocular pain, lacrimation, severe corneal edema. Episcleral injection is usually evident. They can be blinded within 24-72 hours after marked elevation of the IOP. Failure to early recognize clinical signs will lead to permanent blindness. Dogs presented with chronic sign of glaucoma, on the other hand, are quite well tolerant to ocular pain. Chronic increases in IOP results in stretching of the cornea and sclera and enlargement of the globe or buphthalmos (Miller, 2008). Buphthalmic eye (Figure 1) is the cause of other ocular complications such as lagophthalmos, dry eyes and exposure keratopathy (Gelatt et al., 2008).

Early detection of glaucoma provides prompt treatment in glaucoma patients. As the key to protect vision, five tests were recommended by the Glaucoma Research Foundation for glaucoma diagnosis; (1) Measurement of intraocular pressure by tonometer (Figure 2A) (2) Examination of optic disc morphology by using ophthalmoscope (Figure 2B) (3) Visual field test or perimetry to examine the complete of vision (4) Gonioscopy to observe the iridocorneal angle (Figure 2C) and (5) Measurement of central corneal thickness by pachymetry.

Figure 2 Glaucoma diagnostic tests that are available in veterinary practice. Note that

(A) tonometry by rebound tonomter (B) indirect ophthalmoscopic examination to examine optic disc morphology (C) gonioscopic examination to observe iridocorneal angle structure.

Tonometry is the method to measure IOP which minimizes chances of making an important or even catastrophic error in diagnosis (Miller, 2008). Three basic tonometers are commonly used in veterinary practice nowadays; indentation,

applanation and rebound tonometer. Rebound tonometer is gaining its popularity in veterinary practice. It measures IOP by projecting a small probe to the corneal surface and analyzing characteristics of its rebound. Speed of deceleration is measured and converted automatically by the device into pressure of the eye (Martinez-de-la-Casa et al., 2005). It is marketed under the name TonoVet® (Icare Finland, Helsinki, Finland) (Knollinger et al., 2005). Mean IOP in dogs measured by TonoVet® was 16.9 mmHg (SD 3.7 mmHg) (Park et al., 2011).

Treatment

The goal of glaucoma therapy is retention of vision and in order to maintain IOP within a range that will prevent progressive vision loss, IOP should not exceed 20 mmHg in dogs.

Medical treatment of glaucoma

Type and frequency of topical hypotensive medications varies greatly from individual glaucoma patient, as type or severity of the disease. Topical medications for glaucoma treatment until now are classified to five major classes of drug by their active ingredient.

Adrenergic agonists

The two important drugs in this group are Aplaconidine and Brimonidine. Brimonidine is more preferable because it is a more specific agonist to $\alpha 2$ adrenoceptor. It has been shown to produce initial reduction in aqueous humor secretion while increase in uveoscleral outflow for chronic administration. It has been suggested as neuroprotective agent (Shih and Calkins, 2014).

Beta-adrenergic antagonists

Important drugs in this group are timolol, levobunolol, metipranolol, cartiolol and betaxolol. These drugs work by reducing aqueous humor secretion. Timolol and metipranolol have been claimed to be neuroprotective drug of choice (Shih and Calkins, 2014).

Parasympathomimetics

An important drugs in this group are pilocarpine, carbachol and echothiphate iodide. Drugs increase the outflow of aqueous humor through trabecular meshwork by contracting iris sphincter and ciliary muscle, which open trabecular lamellae.

Carbonic anhydrase inhibitors

This drug effectively reduces aqueous humor secretion. Acetazolamide is the first systemic carbonic anhydrase (CA) inhibitors. Useful topical drugs are dorzolamide and brinzolamide.

Prostaglandins analog

Prostaglandins F2α analogs are the newest class of drugs, which are the most effective to lower IOP in humans. Available drugs in this group include latanoprost, travoprost, bimatoprost and tafloprost. The effect on IOP and aqueous humor dynamics of these drugs are similar. They consistently produce substantial increase uveoscleral outflow and to less consistent finding is an increase in trabecular outflow.

Surgical treatment of glaucoma

Glaucoma surgery procedures are classified according to an increase aqueous outflow (e.g. gonioimplantation, filtering procedures) or a decrease of aqueous humor production (transscleral cyclophotocoagulation, endoscopic cyclophotocoagulation, cyclocryosurgery).

Surgery to increase aqueous outflow

Filtering procedure, such as sclerectomy, is performed by creating full or partial thickness holes in the sclera. However, plague by fibrosis over filtering site and long term failure to control IOP in most patients is ususally found.

Gonioimplantation is a procedure that gonioimplants are inserted as artificial aqueous humor shunts to create a drainage pathway for aqueous humor. Problem of development of the scar tissue usually develops and becomes relatively resistant to the flow of aqueous humor.

Surgery to reduce aqueous humor production

Several procedures have been developed to treat glaucoma by decreasing rate of aqueous humor formation by partial destruction of the ciliary body or called cyclodestructive procedure. Using excessive heat, as in lasers, or extreme cold, as in cyclocryotherapy, these energy are directed through the overlying sclera to the ciliary body. Additionally, drugs, such as intraocular gentamicin, intravitreal injected, are extremely toxic to ciliary body epithelium and retina.

Cyclocryotherapy

Cyclocryotherapy (Figure 3) is a procedure that introduces intense cold from either liquid nitrogen or nitrous oxide to the sclera overlying ciliary body. It causes part of ciliary body to be frozen and the ciliary body epithelium to be destroyed by the freeze-thaw cycle. Immediately after freezing, rupture of the ciliary body epithelium and pigmented cells within ciliary stroma occurs. Ciliary body vessels are damaged. Necrosis of ciliary body leads to reduce aqueous production. Complications include the aforementioned IOP spike, uveitis, exposure keratoconjuctivitis, neurotrophic keratitis, hyphema, retinal detachment, recurrence of glaucoma and phthisis bulbi (Assia et al., 1991).

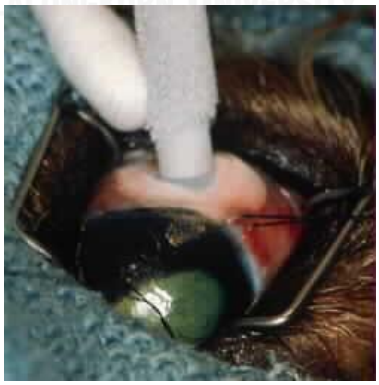

Figure 3 Cyclocryotherapy in glaucoma dog

Cyclophotocoagulation

In the mid-1950s, the first commercial xenon light photocoagulator was generated, followed by the ruby laser with a pulse of red in the early 1960s and the blue-green argon laser in the late 1960s. By the early 1990s, Nd:YAG laser with green light was developed (Munnerlyn, 2003) and then diode laser which was first operated in 1962. The earlier laser units, such as argon, krypton, and the first Nd:YAG laser were not really suitable for the use in veterinary medicine because they were large and heavy units requiring elaborate cooling system (Spiess, 2012). The advent of semiconductor diode lasers has made laser affordable for veterinary practice (Pablo et al., 1996).

More recently, cyclophotocoagulation has become more popular in glaucoma treatment. It involves three basic tissue-laser interactions which are photocoagulation, photodisruption and photoablation (Munnerlyn, 2003). With photothermal effects, heat energy is deposited in the tissue by the absorption of light and its subsequence conversion to heat via collisional relaxation. This causes a rise in temperature of the tissue. After the heat has diffused through the tissue, it causes a rise in temperature in the surrounding tissue. Damage done to the tissue depends on the temperature that is reached, and the duration at which it is held at that temperature (Niemz, 2013). Ciliary processes are target tissues destroyed by laser energy, which in turns resulting in reduction of the IOP.

Most common techniques of cyclophotocoagulation being used in veterinary ophthalmology are transscleral cyclophotocoagulation (TSCP) and endoscopic cyclophotocoagulation (ECP). ECP (Figure 4) is a direct method to coagulate ciliary body under endoscopic outline. ECP offers the advantage of highly selective laser ablation of pigmented ciliary body epithelium via direct visualization (Bras et al., 2005). It requires low amount of energy, therefore, inflammation is reduced (Lin et al., 2006). However, it is considered an invasive technique that requires surgical theater during performance. Potential risk of damage to crystalline lens is high as well as risk of ocular infection (Mandal et al., 2009).

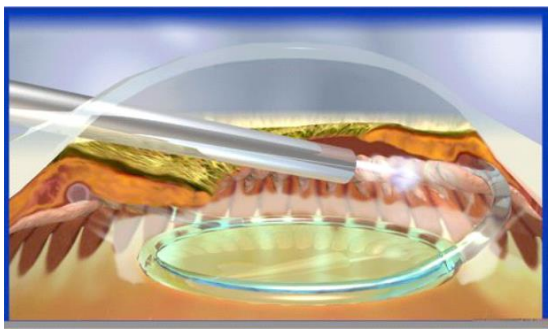

Figure 4 Schematic picture showed endoscopic cyclophotocoagulation procedure

TSCP, on the other hand, is a noninvasive method by which laser light is transmitted through a direct contact at the sclera (Figure 5). Laser energy is absorbed by melanin pigments in the outer layer of ciliary body epithelium (Pantcheva et al., 2007a). There are currently two available types of laser for TSCP; Nd:YAG laser (1064 nm wavelength) and semiconductor diode laser (810 nm wavelength). Both types of laser can produce thermal tissue damage. However, as a comparison to diode laser, Nd:YAG laser introduced more destruction of collateral tissues and more inflammation around the laser sites (Schuman et al., 1990).

Diode laser has gained popularity in veterinary practice, as many reported studies in glaucoma treatment in dogs (Hardman and Stanley, 2001) and horses (Gemensky-Metzler et al., 2014). It is selectively absorbed by melanin pigment. Transscleral cyclodiode therapy is usually applied to ciliary body with the use of a contact G-probe at posterior distance to the corneoscleral limbus. The fiber-optic tip on the contact probe indents conjunctiva and sclera during treatment, thus enabling more effective laser transmission to the underlying ciliary body. Rising of temperature within target tissues to 42-60°C, photoablation occurs (Berger and Eeg, 2008). Blood vessels are contracted and destroyed causing tissue hypoxia and cell death. As tissue temperature increases to 60-100°C, they are undergone photocoagulation, by which collagen fibers contract and proteins denature. When tissue is overheated with temperature above 100°C, boiling of tissue water produces uveal micro-explosion (photodisruption) of ciliary processes (Lin et al., 2006). Shockwave created from overheated tissue then results in the “pop” sound (Prum et al., 1992).

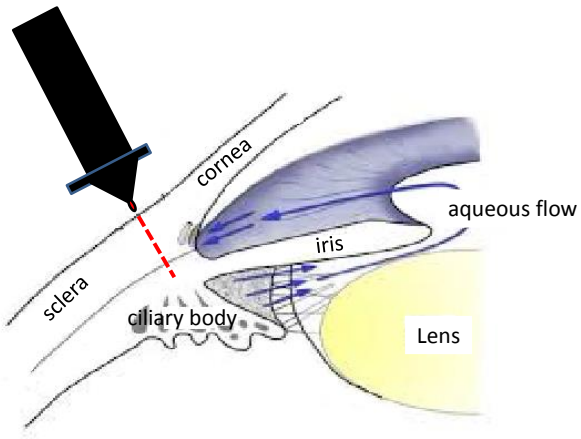

Figure 5 Schematic diagram illustrated transscleral cyclophotocoagulation procedure. Placement of probe on the sclera with laser beam aim to ciliary processes.

Suitable laser energy setting and appropriate site of laser application have come into consideration for a success of TSCP. There is no widely acceptable standardized treatment protocol and the optimal laser settings of TSCP is still unpredictable. Setting of laser energy significantly depends on laser power (milliwatt; mW) together with exposure time (millisecond, ms), number of laser spots, energy per laser application (Joule = watt x second) and total energy delivery per eye (Joule = watt x second x number of laser sites).

A few studies have specifically investigated whether the laser power alone has an effect on the effectiveness of lowering IOP, but no significant difference in the final mean IOP is found (Shahid, 2013). On the other hand, direct linear correlation between the amount of total energy applied to the ciliary body and the percentage of patient with successful outcome was reported (Hauber and Scherer, 2002).

Several studies showed an attempt to achieve high success rate in reducing the IOP. Laser setting of 1,000 mW for 5,000 ms at 25 laser spots resulting in 125 Joules of total energy delivered per eye revealed 92% reduction of IOP to below 25 mmHg in canine primary glaucoma (Hardman and Stanley, 2001). However, eyes with highly elevating IOP after TSCP of this particular study, either paracentesis were considerably performed or TSCP of the same setting was repeated. Ulcerative keratitis and cataract were major complications (Hardman and Stanley, 2001). Rather aggressive setting of

2,250 mW for 2,000 ms for 20-28 laser spots achieved 61% success in IOP control of advanced glaucoma in human without retreatment. Though, intraocular hemorrhage, severe uveitis and sterile hypopyon were observed (Noureddin et al., 2006). A setting of low power (1,000 mW) with short exposure time (1,500 ms) for 20-25 laser spots provided 50% reduction of IOP in refractory glaucoma in human. Although, clinical complications were not apparent, hypotensive medication could not be discontinued (Mahmood et al., 2011).

In veterinary practice, a setting of 1,500 mW and 1,500 ms for up to 36 laser sites was recommended in animals (Spiess, 2012). From this laser protocol, Morreale et al. (2007) revealed coagulative necrosis pattern without alteration of ciliary processes architecture from histological tissue sections. Vascular effect was demonstrated as vascular congestion, thrombosis (Lin et al., 2006) as well as capillary destruction in ciliary processes and ciliary muscle (McKelvie and Walland, 2002). Similar destruction of pigmented and non-pigmented ciliary epithelium and capillaries were also reported in TSCP-treated glaucoma patients (Pantcheva et al., 2007a). Although cyclodestruction from diode laser is focally focused on pars plicata, epithelial cells loss was noted. It was postulated that position of ciliary body is non-uniform among glaucoma subjects. Even though destruction of pars plana was believed to enhance reduction of IOP by triggering uveoscleral outflow, failure to long-term control IOP occurred (Walland and McKelvie, 1998).

Extensive irreversible destruction of the ciliary processes from TSCP is desirable in some way but yet it may increase risk of complications. Unacceptably high rate of complications; such as ocular hypotony, hyphema and ocular pain were reported in glaucoma patients treated with TSCP setting of 1,750 mW and 2,000 ms for 18 laser spots (Kaushik et al., 2008). Malignant glaucoma was noticed after the use of aggressive TSCP protocol (Azuara-Blanco and Dua, 1999). Pre-iridal fibrovascular membrane that was evident along the iris after severe inflammation may be an underlying cause of intraocular hemorrhage following aggressive laser therapy (Zarfoss et al., 2010).

To achieve the suitable protocol for TSCP, in human, the influence of total energy delivery on success rate after TSCP has become considerable. The result of this particular study demonstrated a significant, direct linear correlation between the total

amount of energy applied to the ciliary body and the percentage of patients with a successful outcome (Hauber and Scherer, 2002). Similarly in animal study, there was dose-response-relationship studied between number of laser applications and IOP reduction in Chinchilla bastard rabbits which showed positive relationship between difference of IOP (pre- and post-treatment) and the number of laser burns (Wagenfeld et al., 2014). Therefore, the study of relationship of laser setting parameters can possibly provide more information about optimal TSCP protocol and appropriate laser applications for canine glaucoma.

According to the fact that different degree of response to glaucoma treatment by using diode laser can be varied by many factors; laser setting parameters, position of laser applications and this might including breeds predisposing. Newkirk et al. (2010) found difference in distribution and amount of melanin pigment within ciliary body of dogs with blue and brown iris. Dogs with blue iris lack of melanin pigment around the ciliary body musculature, but comparable amounts in the ciliary processes. The presence of pigment within target tissues is necessary to achieve effective tissue destruction.

Histopathological changes of ciliary body after received diode laser TSCP were reported in many species; such as, human (Pantcheva et al., 2007a), horses (Morreale et al., 2007), rabbit (Brancato et al., 1991), pigs (Pantcheva et al., 2007b) and dogs (Nadelstein et al., 1997). Pantcheva et al. (2007a) studied acute changes of porcine ciliary processes after TSCP. Tissue being treated with TSCP showed pronounced tissue disruption of both pigmented and non-pigmented ciliary epithelium, ciliary capillaries in ciliary processes with coagulative damage and destruction of the stroma, extending to ciliary muscle. It is reported in the post-mortem studies of human and porcine eyes that pars plana burns after TSCP. This created passive pars plana transscleral flow thereby increased uveoscleral outflow (Schubert, 1989). Ho et al. (2002) performed a study of diode laser transscleral pars plana photocoagulation in human glaucoma. They found significant reduction of the IOP.

Application of special stains in addition to routine H&E will assist investigation of pathological changes in pars plicata and pars plana whether it is morphologically influenced by diode laser. Special staining, such as Periodic acid Schiff (PAS), Masson's

trichrome and Masson's Fontana, is useful to detect morphological changes in particular tissues. Disorganization of collagens and smooth muscles of ciliary body were detected with Masson's trichrome staining in experimental rabbits treated with cyclocryotherapy. PAS staining was performed to locate ciliary body epithelium in canine glaucomatous eyes (Reilly et al., 2005), as well as to identify non-pigmented ciliary epithelium in humans treated with TSCP (Mahmood et al., 2011). Masson's Fontana was stained to identify the melanin pigments-rich area in ocular organ, especially in ciliary body; the target tissue for TSCP (Newkirk et al., 2010). However, application of special stains to reveal morphological changes of ciliary body has not yet scientifically reported in canine glaucoma treated with TSCP.

Three different settings of TSCP protocols are created as the treatment for canine chronic glaucoma with various laser parameters. These three TSCP protocols were designed to seek for an effective control of IOP while minimizing degrees of clinical complications. Correlation between laser setting parameters of each protocol and clinical outcomes was investigated.

Chapter 3

Materials and methods

Animals

Forty-two glaucomatous eyes from 37 dogs diagnosed with chronic canine glaucoma were included into this study (Figure 6). These dogs were undergone glaucoma therapy at the Ophthalmology clinic, Small Animal Teaching Hospital, Faculty of Veterinary Science, Chulalongkorn University. They had reached the following 5 important criteria: (1) uncontrolled high IOP over 2 months duration (2) IOP higher than 30 mmHg, (3) unresponsive to topical hypotensive medications, (4) no previous surgical treatment for glaucoma and (5) no specific ophthalmic complications, such as hyphema or intraocular infection.

All dogs had ophthalmic examinations including neuro-ophthalmic investigations (menace response, dazzle reflex, pupillary light response and palpebral reflex), Schirmer tear test I, fluorescein staining, intraocular pressure measurement using a rebound tonometer (Tonovet®; Icare Finland Oy, Vantaa, Finland) and anterior chamber examination with the use of slit lamp biomicroscope (Kowa SL-15®; Kowa company. Ltd., Shizuoka, Japan). All procedures were performed under informed consent provided by owners of the dogs. Experimental procedures were approved by Chulalongkorn University Animal Care and Use Committee, Bangkok, Thailand (No. 1531067).

Figure 6 Chronic advance glaucoma in a dog showed buphthalmic eye, episcleral vessels congestion and severe exposure keratitis.

Procedures

This study was divided into 2 parts.

Part1: Investigation of clinical outcomes after receiving different TSCP protocols

Part2: Study of pathological changes of ciliary body following different TSCP protocols.

Part 1: Investigation of clinical outcomes after receiving three different TSCP protocols

Sample collection

Animal preparation Thirty-three chronic glaucomatous eyes from 27 dogs were included in this part. Dogs had undergone thorough physical examinations, blood collection to evaluate health status (complete blood count, blood urea nitrogen (BUN), Creatinine, alanine transaminase (ALT), alkaline phosphatase (ALP), total protein and albumin level). Dogs those were older than 7 years of age were requested to have thoracic radiography and electrocardiography to ensure good cardiac function before TSCP.

Three days before TSCP, dogs had been topically receiving 0.3% tobramycin eye drops (Tobrex® ophthalmic solution; Alcon-Couvreur, Puurs, Belgium) for four times daily, together with 0.5 mg/kg prednisolone orally twice daily.

On the day of TSCP, all dogs were performed thorough physical and ophthalmic examinations (as described above) prior to general anesthesia. Intraocular pressure was measured using a rebound tonometer. B-scan ocular ultrasonography was performed using ocular ultrasonographic machine (Ultrascan® Imaging system; Alcon Laboratories, Inc, Hünenberg, Switzerland). From B-scan ultrasonographic image, axial globe length (millimeter) was electronically measured along visual axis from cornea to the posterior wall of the eye.

Anesthetic procedure Dogs were withheld water and food for 6 and 12 hours, respectively, prior to general anesthesia. 25 mg/kg cephazolin sodium (Zefa M.H. ®; M&H Manufacturing. Co. Ltd., Samutprakarn, Thailand) and 0.5 mg/kg dexamethasone sodium phosphate (Lodexa®; L.B.S. Laboratory Ltd., Bangkok, Thailand) were systemically given to all dogs. Dogs were premedicated with 0.03 mg/kg acepromazine maleate (Combistress®; Phenix Pharmaceutical N.V., Antwerp, Belgium) combined with 0.3 mg/kg morphine sulfate intramuscularly. General anesthesia was induced with 4-6 mg/kg propofol (Lipuro®; B.Braun, Melsungen, Germany) intravenously. Level of anesthesia was maintained with 2-2.5% isoflurane (Aerrane Isoflurane USP®; Baxter Healthcare of Puerto Rico, Puerto Rico) delivered in oxygen at a flow rate of 200 ml/kg. Lactate Ringer's Solution was given intravenously at the rate of 5-10 ml/kg/hr throughout anesthesia and until fully recovered.

Transscleral diode laser cyclophotocoagulation procedure

After general anesthesia dogs were arranged in lateral recumbency position with glaucomatous eye upward. Excessive hairs around the eye were clipped as necessary. Eye was cleansed and disinfected with Lugol's solution. In order to keep the eyeball in primary gaze, fixation forceps were placed at 3 and 9 o'clock position of the globe, where long ciliary artery runs along. TSCP was performed using an 810-nm diode laser unit (DioVet Laser System®; IRIDEX, Mountain View, USA) with laser energy being delivered via glaucoma probe (G-probe) (Figure 7). Thirty-three chronic glaucomatous eyes were randomly assigned into 3 groups (two groups, n=10, and one group n=13) of different setting protocols of TSCP (Table1). Important TSCP factors were laser power (milliWatt; mW), exposure laser time (millisecond; ms), number of

laser spots, degree circumferential of the globe in accordance with laser spots (Figure 8) and total energy delivery (Joules per eye).

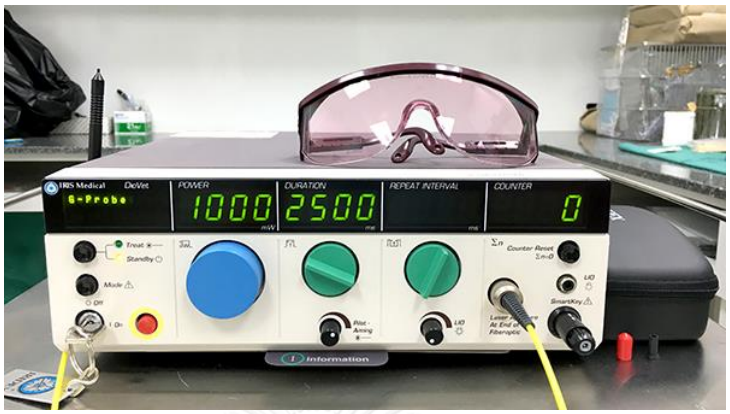

Figure 7 Transscleral diode laser cyclophotocoagulation machine (810-nm diode laser).

Protocol	Laser power (mW)	Exposure Time (ms)	Number of laser spot	Degree circumferential	Total energy Delivery (Joule)
I	1500	1500	40	270	90
II	1500	1500	80	270	180
III	1000	2500	80	360	200

Table 1 Three different setting of TSCP protocols

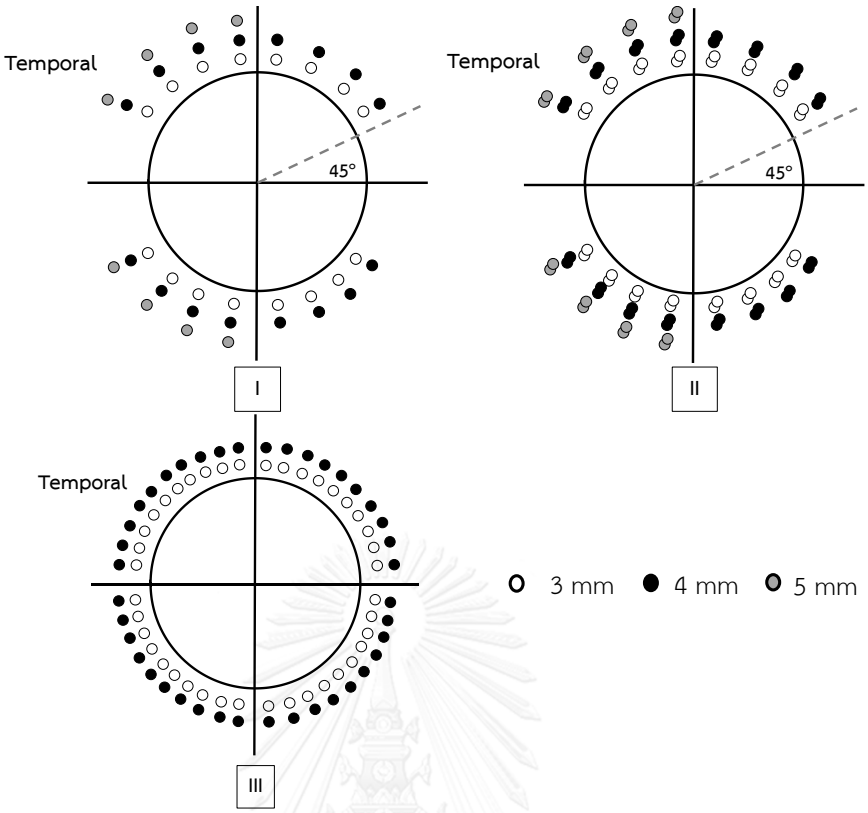

Figure 8 Three schematic diagrams demonstrating different laser positions posteriorly to the limbus. Note that protocol I at 3 and 4 mm (4 positions in each row); 5 mm (4 positions), protocol II at the same positions as described in protocol I but in repeated manner at the area next to the previous position, protocol III at 3 and 4 mm (10 positions in each row).

G-probe was placed perpendicularly to the sclera. Lasers were applied at different distances posteriorly to the limbus as designed in each protocol. Measurement of IOP was repeated immediately after TSCP. If the IOP was above 20 mmHg, anterior chamber paracentesis was considered to release ocular tension to the level of 20 mmHg. Subconjunctival injection of 0.8 mg gentamicin sulfate combined with 2 mg dexamethasone sodium phosphate was given to all dogs. Monitoring of vital signs was continued until dogs fully recovered. Ocular examinations using transilluminator or slit-lamp biomicroscope were repeated to observe any abnormalities that may have occurred following TSCP.

Figure 9 TSCP procedure. (A) Dog with chronic canine glaucoma of left eye, (B) dog undergone general anesthesia and posed in lateral recumbency with affected eye upward, (C) globe stably fixed with fixation forceps at 3 and 9 o'clock together with additional forceps as needed, and (D) laser application.

All dogs were given 25 mg/kg/day cefazolin sodium and 0.5 mg/kg/day dexamethasone sodium phosphate subcutaneously for 4 consecutive days after TSCP. Dexamethasone sodium phosphate was thereafter be replaced by 0.5 mg/kg prednisolone orally for 2 weeks with a withdrawing dose. All topical hypotensive medications were continued then application frequency re-adjusted regarding to post-TSCP IOP values.

Data collection and analysis

Seven parameters were investigated and analyzed.

1. Audible “pop” sounds: Audible “pop” sounds observed during TSCP were recorded. Number of “pop” sounds was analyzed in percentage in each TSCP protocol. Statistical comparison was performed with the use of repeated measured one-way analysis of variance (ANOVA) with a significant level at $p<0.05$ using SPSS program version 22 (IBM Corporation, NY, USA).
2. Intraocular pressure: IOP was measured before TSCP, immediately after TSCP and post TSCP at day 1, 2, 3, 4, 7, 14, 21, 28, 56, 77 and 105. Mean IOP and standard deviation (SD) were calculated at all time points. Repeated measured one-way analysis of variance was applied to compare mean IOP among each time points, with a significant level at $p<0.05$ using SPSS program version 22 (IBM Corporation, NY, USA).
3. Number of topical hypotensive drug administration: Number of topical hypotensive eye drop applied to each eye was recorded before and after TSCP. Numbers of administration were calculated into mean ± SD and statistically compared among each time points of clinical follow-up. Repeated measures one-way analysis of variance was applied with a significant level at $p<0.05$, using SPSS version 22 (IBM corporation, NY, USA).
4. Axial globe length: AGL at before TSCP and post TSCP at day 28, 56 and 105 was measured along visual axis from the cornea to the posterior wall of the eye. Mean AGL and SD were calculated. Repeated measured one-way analysis of variance was applied to compare mean AGL among each time points, with a significant level at $p<0.05$ using SPSS program version 22 (IBM Corporation, NY, USA).
5. Success of TSCP to control IOP: Qualified success (percentage) was defined when the final IOP was less than or equal to 21 mmHg (Wilson, 1997) with continued topical hypotensive medication.
6. Ocular complications: Post TSCP complications were recorded at all time points. They were descriptively analyzed in percentage in each TSCP protocol.

7. Correlation study: Pearson's correlation was used to study relationship between total energy delivery and clinical outcomes and regression analysis was studied for estimating the relationships among total amount of energy, clinical outcomes of each TSCP protocol and post TSCP ocular complications using SPSS program version 22 (IBM Corporation, NY, USA), with a significant level at $p<0.05$.

Part 2: Study of pathological changes of ciliary body following different TSCP protocols.

Sample collection

Animals Eleven eyes from 11 dogs were included in part 2 of the study. All dogs, whose owners decided to treat ocular disorder by enucleation, were from Ophthalmology clinic, Small Animal Hospital, Faculty of Veterinary Science, Chulalongkorn University.

All eyes were divided into 3 groups. (1) Negative controls (2 eyes) derived from non-glaucoma dogs; (2) positive controls (3 eyes) derived from chronic glaucoma without any previous surgical treatment; and (3) experimental groups (6 eyes) derived from dogs undergone TSCP. In the experiment group, three eyes were collected immediately after TSCP of each protocol. The other three eyes were post TSCP samples derived from protocol I (78 and 360 days) and protocol II (11 days)

Procedure to remove the eyeball All dogs were under general anesthesia (as described in part1). All eyes were removed by transpalpebral enucleation technique (Figure 10). Dogs were positioned in lateral recumbency with an affected eye upward. Following standard aseptic technique, periocular area and ocular surface were prepared. Transpalpebral incision was created in elliptical fashion. Blunt dissection of conjunctiva and Tenon's capsule followed by transection of extraocular muscles were performed. Once approaching toward the posterior wall of eyeball, optic nerve was transected, eyeball was then removed. Subcutaneous tissues were closed with 3-0 absorbable suture in simple continuous pattern, followed by a closure of skin with 2-0 nonabsorbable suture in simple interrupted pattern. Post-operative pain was control with 4 mg/kg tramadol hydrochloride (Tramal®; Aayush Food&Herbs limited, Karnataka, India) orally for up to 3 days after surgery. 25 mg/kg cephalexin monohydrate (Sialexin®; Siam Bheasach, Bangkok, Thailand) had been orally administered for 7 days after surgery. Skin sutures were removed on day 10 postoperatively.

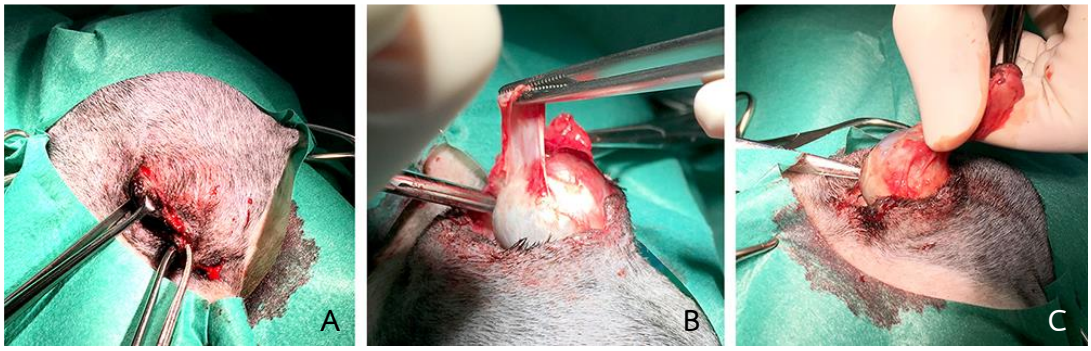

Figure 10 Transpalpebral enucleation technique. (A) Transpalpebral incision was created through eyelid. (B) Extraocular tissues around the eyeball were dissected. (C) Optic nerve was transected to freely remove an eyeball from a socket.

Pathological procedure To maintain good quality of intraocular structures, small amount of 4% paraformaldehyde in 0.1M PBS was injected via 26G needle into the vitreous. After extraocular muscles around the globe were removed, globe was then immersed into the same fixative at 4ºC overnight. Thereafter, globe was dissected with a sharp razor blade through a sagittal plane into 2 halves (Figure 11B). Vitreous and lens were gently discarded. Another sagittal plane was cut to divide each half into 2 pieces (Figure 11C). With the aid of bright light originated from transilluminator, area of ora serrata was identified; white area that had been exposed to laser was observed to be selected. Two cuts were made though coronal plane to get rid of the unnecessary far most anterior and posterior of the eyecup. Area of ciliary body that was comprised of the beginning of pars plicata until the end of pars plana was then selected and shortly submitted for pathological study (Figure 11F).

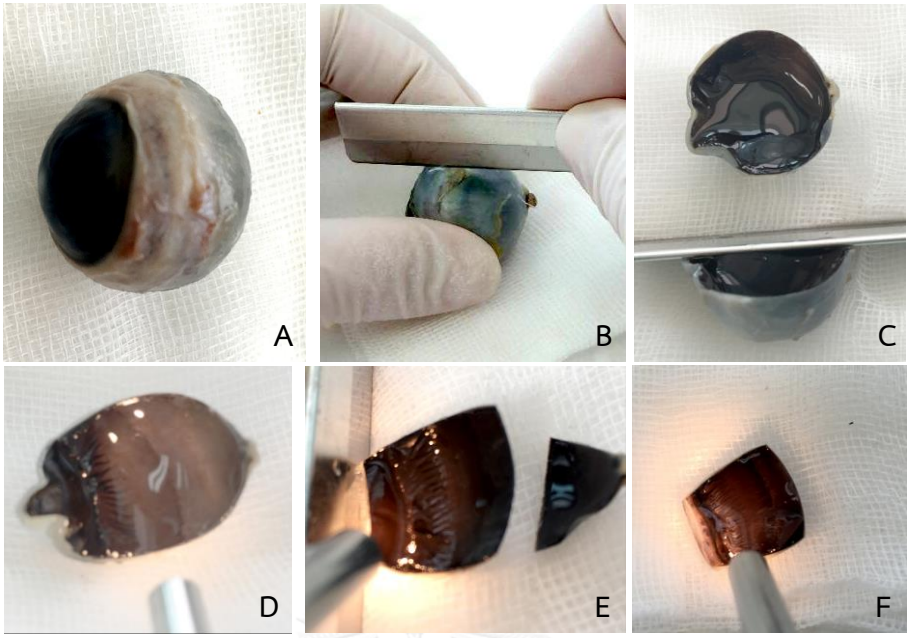

Figure 11 Histopathological sectioning. (A) Excessive extraocular muscles were dissected from enucleated eyeball. (B) Globe was cut via sagittal plane with a razor blade. (C) Lens and vitreous were removed from the sections. (D) After each section was additionally cut through sagittal plane, ciliary body was identified. (E) Posterior pole of the globe behind ora serrata was discarded, while anterior pole before the beginning of pars plicata was discarded. (F) Area that had been introduced with diode laser, turning white color, was selected for tissue processing.

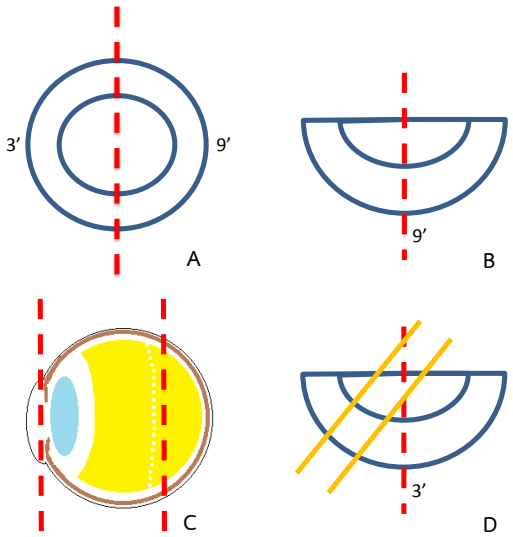

Figure 12 Schematic diagram of tissue sectioning. (A) Sagittal cut (anterior to posterior) was made to create 2 halves of eyeball. (B) Each half of eyeball was cut through sagittal plane. (C) Area that ciliary body was located and specifically selected by discarding unnecessary portions through two additional cuts. (D) Area that ciliary process was introduced with diode laser was selected.

Data collection and analysis

Tissue sections were histologically processed, embedded in paraffin, cut into 4 μm thickness and stained with Hematoxylin and Eosin (H&E) for light microscopic investigation. To investigate microstructural changes of the ciliary body, deparafinization was performed followed by special staining; Periodic Acid Schiff (PAS), Masson's trichrome and Masson's Fontana. Pathological changes of pars plicata, pars plana and surrounding area were microscopically investigated and compared among group of experiment by descriptive manner.

CHAPTER 4

RESULTS

Part 1: Investigation of clinical outcomes after receiving different TSCP protocols

Animals

Thirty –three chronic glaucomatous eyes were from 26 dogs of various breeds; Shih Tzu (n=10), Poodle (n=7), Mixed breed (n=6), Pug (n=1), Chows (n=1) and Chihuahua (n=1). Both genders were equally in number (male=13, female=13). Average age of dog was 9.77 years which was ranged from 3 to 15 years. TSCP was performed on the right eye in 5 dogs, left eye in 14 dogs and both eyes in 7 dogs (Table 2).

Breed	Left	Right	Both
Shih Tzu	3	2	5
Poodle	5	1	1
Mixed	5	-	1
Pug	-	1	-
Chows	1	-	-
Chihuahua	-	1	-

Table 2 Number of eyes from various breeds included in this study.

1. Audible "pop" sound

Mean percentages of pops sound were 67.50±17.48 (55.00-80.00 95%CI), 66.09±17.17 (53.74-78.45 95%CI) and 54.62±14.11 (46.09-63.14 95%CI) in protocol I, II and III, respectively (Figure 13). Statistically significant difference was not evident among three TSCP protocols.

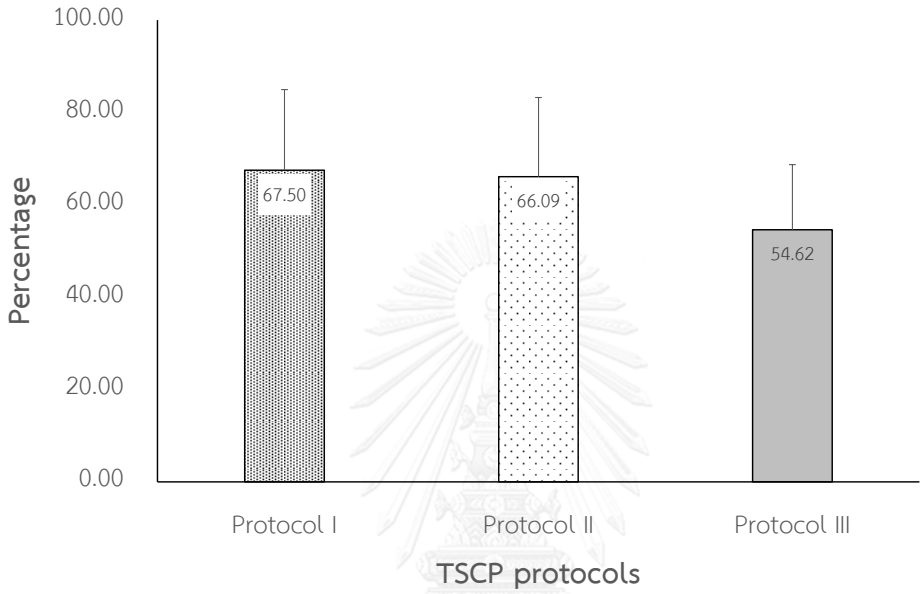

Figure 13 Percentage of audible "pop" sounds of each TSCP protocol.

2. Intraocular pressure

Protocol I (Figure 14)

IOP was successfully controlled to a level of below 21 mmHg in six out of ten eyes (60%). Mean IOP values apparently decreased within 2 days after TSCP from 56.65±13.87 (46.73-66.57 95%CI) to 24.00±16.31 mmHg (11.46-36.54 95%CI). They were then maintained at level slightly above 21 mmHg for another 2 days before continue decreasing to 12.00±10.70 mmHg (0.77-23.23 95%CI) at day 7 post TSCP. Mean IOP was raised and sustained from day 56-77 then lowered to 15.10±9.30 mmHg (3.55-26.65 95%CI) at the end of the study. Significant differences of the mean IOP between pre and post TSCP period had been found since day 2 onwards. Mean IOP difference between pre TSCP period and at the end of the study was 41.55 mmHg (9.45-73.65 95%CI, *p=0.001).*

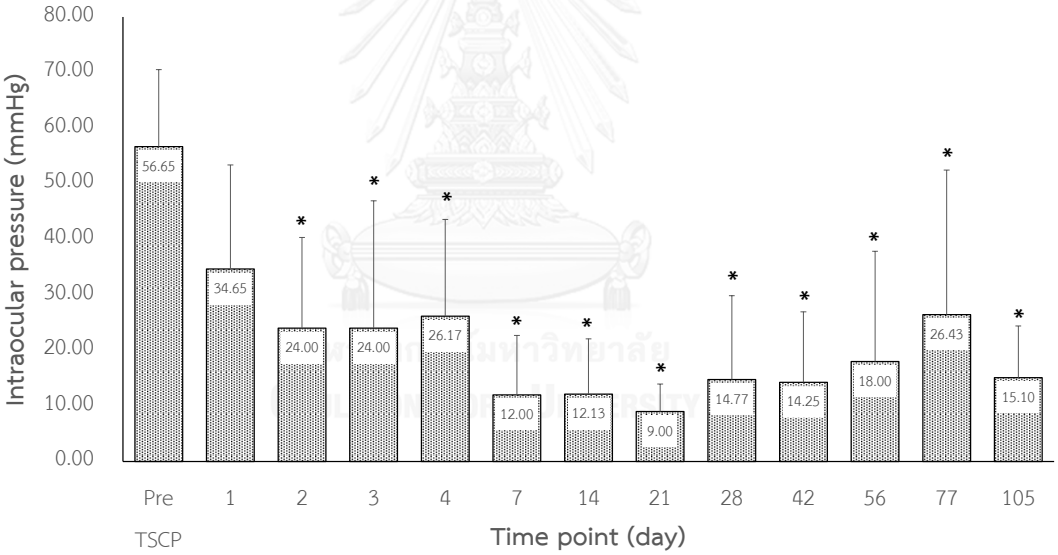

Figure 14 Mean intraocular pressure (mmHg) ± SD from **protocol I** at pre/post TSCP. Asterisk (*) indicates significantly difference of IOP reduction at *p<0.05*).

One out of four eyes that failed to respond to TSCP protocol I finally had mean IOP lower than 21 mmHg at day 119; after the end of the study. Another three eyes turned out to be enucleated. IOP of two eyes was unresponsive to TSCP, in the meantime, intraocular complications developed. There were deep ulcerative keratitis, together with aqueous flare and hypopyon (Figure 15). IOP of the other eye was initially

under controlled at a level of 15 mmHg (day 56 post TSCP). It abruptly increased to above 60 mmHg at day 69 and maintained at extremely high level until day 105.

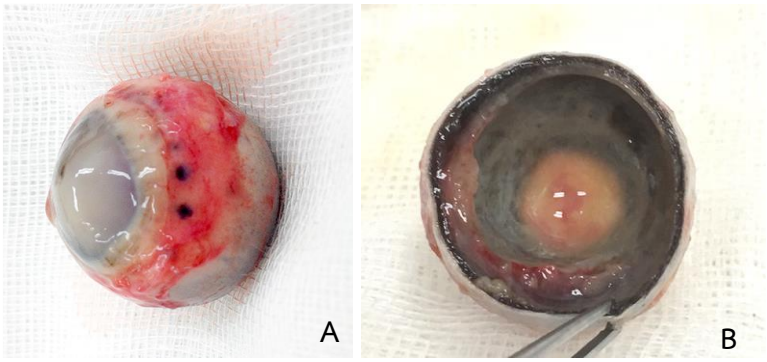

Figure 15 Photographs of para TSCP complications of protocol I. (A) Side view of the eyeball revealed serious inflammation around laser locations and aqueous accumulation. (B) Anterior eyecup revealing intraocular infections and bleeding.

Protocol II (Figure 16)

IOP was successfully controlled to a level of below 21 mmHg in nine out of ten eyes (90%). Mean IOP had significantly decreased since day 1 post TSCP with the mean IOP difference of 28.85 mmHg (12.84-44.86 95%CI, *p=0.001*). Mean IOP continued to decrease and maintain at a level lower than 21 mmHg from day 2 post TSCP until the end of the study. Statistically significant differences between pre and post TSCP were observed at all follow up time points (*p<0.05*). Mean IOP at day 105 post TSCP was 7.00±6.70 mmHg (1.32-15.32 95%CI). The one unsuccessful eye was enucleated at day 11 post TSCP due to increasing IOP associated with deep ulcerative keratitis and hypopyon (Figure 17).

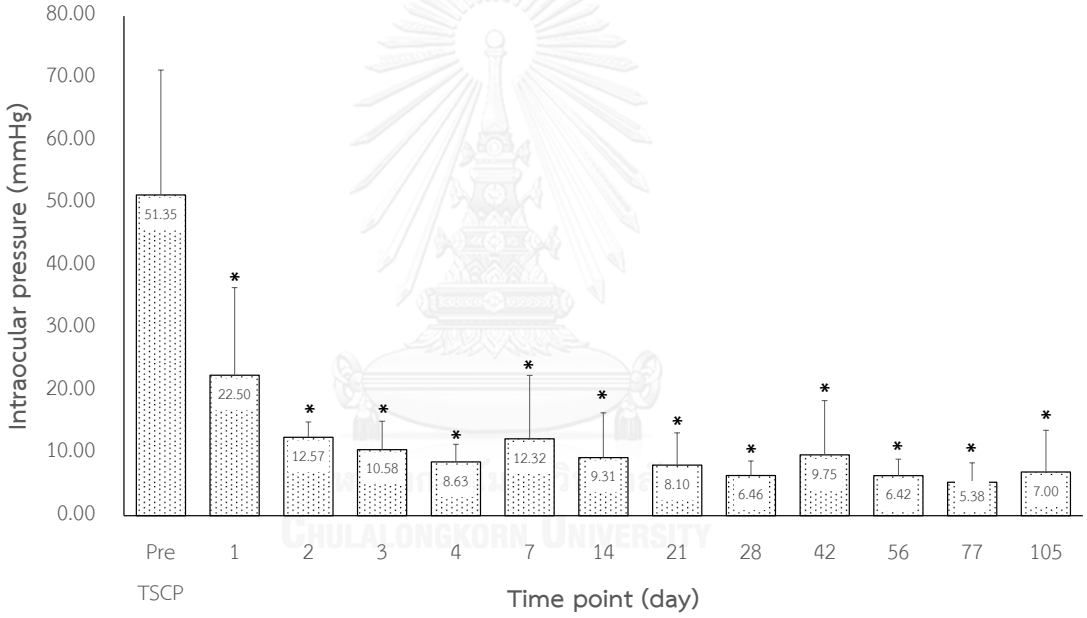

Figure 16 Mean intraocular pressure (mmHg) ± SD from **protocol II** at pre/post TSCP. Asterisk (*) indicates significantly difference of IOP reduction at *p<0.05*).

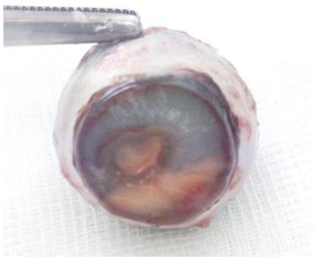

Figure 17 Photographs of para TSCP complications of **protocol II**.

Protocol III (Figure 18)

All eyes (n=13) receiving TSCP protocol III succeeded in lowering IOP below the cut point level by the end of the study (100%). Mean IOP had significantly decreased since day 1 post TSCP, at which statistical difference as compared to pre TSCP was indicated (*p=0.001*). Mean IOP continued to decrease by time throughout the study. It was 4.83±1.76 mmHg (0.47-9.20 95%CI) at the end of the study with a significant level at *p=0.001* as compared to pre TSCP value.

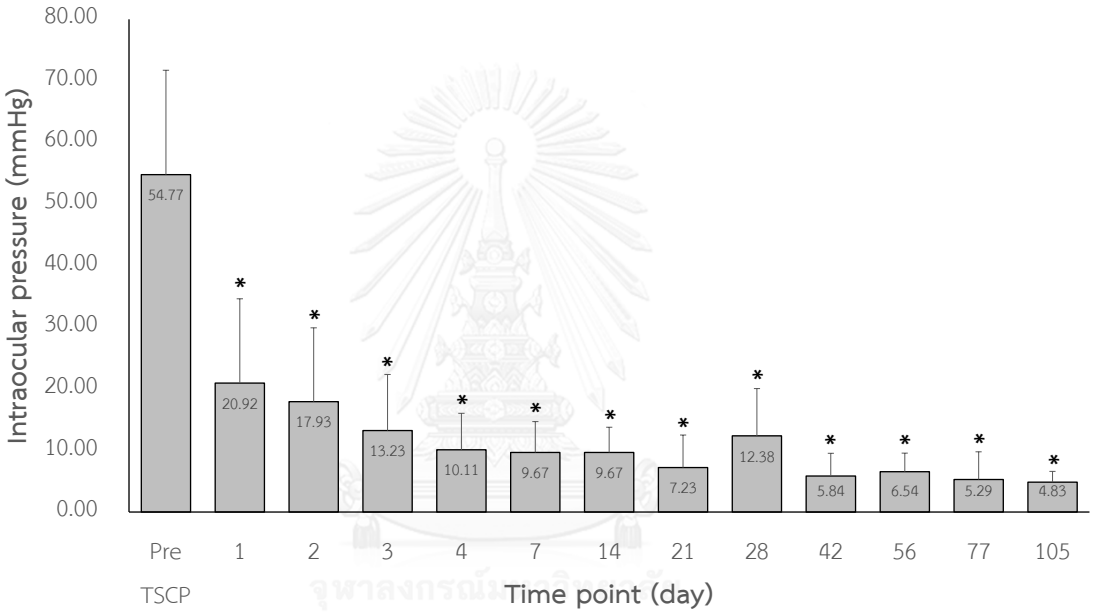

Figure 18 Mean intraocular pressure (mmHg) ± SD from **protocol III** at pre/post TSCP. Asterisk (*) indicates significantly difference of IOP reduction at *p<0.05*).

Comparison of IOP difference among three TSCP protocols revealed statistical difference between protocol I and III at day 4 and 56 post TSCP (*p<0.05*) (Figure 19).

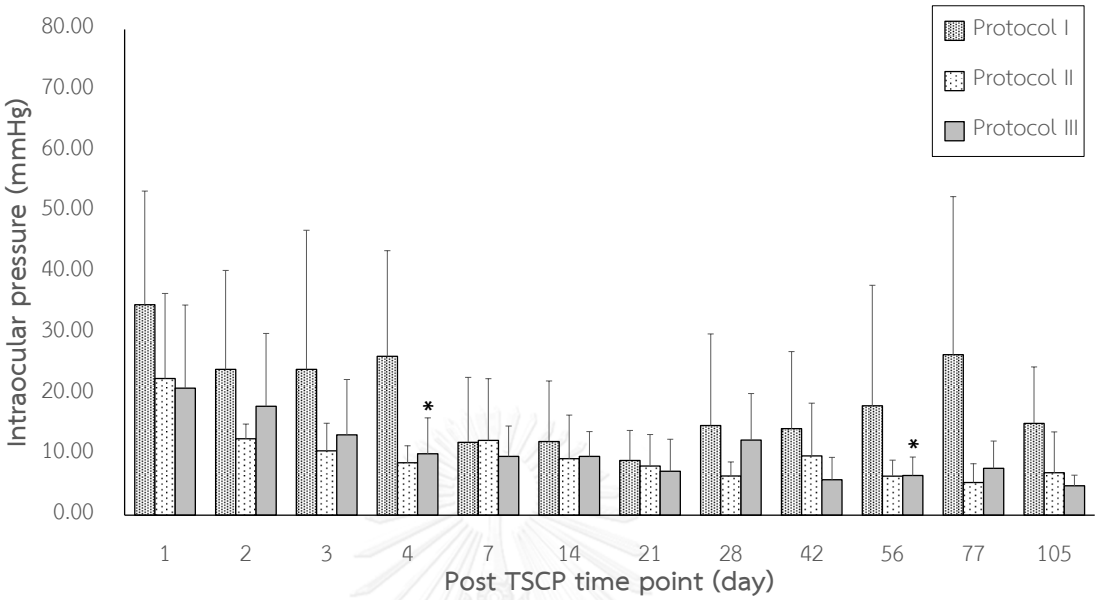

Figure 19 Mean intraocular pressure (mmHg) ± SD from protocol I, II and III post TSCP. Asterisk (*) indicates significant difference of IOP reduction between protocol I and III at *p<0.05*.

	Protocol I (mean,SD)	Protocol II (mean,SD)	Protocol III (mean,SD)
Baseline IOP (95% CI)	56.65,13.87 (46.73,66.57)	51.35,20.00 (37.04,65.66)	54.77,16.96 (44.52,65.02)
Final IOP (95% CI)	15.10,9.30 (3.55,26.65)	7.00,6.70 (1.32,15.32)	4.83,1.76 (0.47,9.20)
% IOP reduction at first day post TSCP	38.83%	56.18%	61.80%
% final IOP reduction	73.34%	86.36%	91.18%
Mean day of maximal IOP reduction	21	77	105
Qualified success (number of eye)	6/10	9/10	13/13
Complete success (number of eye)	0/6	1/9	1/13

Table 3 Clinical outcome of IOP after received three different setting TSCP protocols

3. Topical hypotensive medications

Topical hypotensive drugs those were applied to all dogs included α_2-adrenergic agonists, β-blockers, carbonic anhydrase inhibitors and prostaglandin analogs. Type of topical hypotensive drugs prescribed before TSCP was continued as they were in each dog individually.

Protocol I (Figure 20)

Mean number of topical hypotensive applications before TSCP was 9.60±3.10 drops per day (7.38-11.82 95%CI). They were ranged from 6-16 drops per day. After TSCP, mean eye drops were relatively maintained during the first 14 days, and then gradually reduced to 7.73±2.15 drops (6.28-9.17 95%CI) on day 28. Mean number of drop had been maintained until post TSCP day 77. It obviously decreased on day 105 which was 5.57±5.09 drops per day (0.86-10.28 95%CI). Statistically significant difference was not showed.

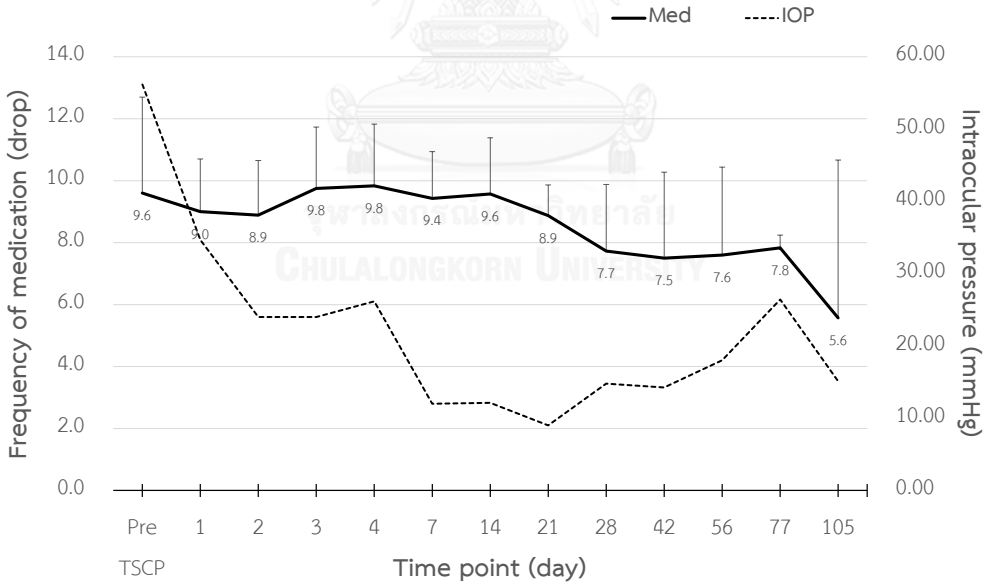

Figure 20 Mean number of topical hypotensive application (drops) per day ± SD at pre/post TSCP **protocol I**.

Protocol II (Figure 21)

Mean number of topical hypotensive applications was 8.78±2.73 drops per day (6.68-10.87 95%CI) before TSCP. Number of drops was ranged from 4-12 drops per day. After TSCP, mean number of topical hypotensive eye drops had been sustained at the same level as that of pre TSCP for the first 7 days. Gradual reduction of application frequency was then observed from that moment until the end of the study. Mean number of topical eye drops was 2.92±2.91 drops per day (0.13-5.97 95%CI). Statistical difference of mean number of eye drops was found since day 56 post TSCP onwards. One out of ten eyes no longer required topical medications at the end of the study.

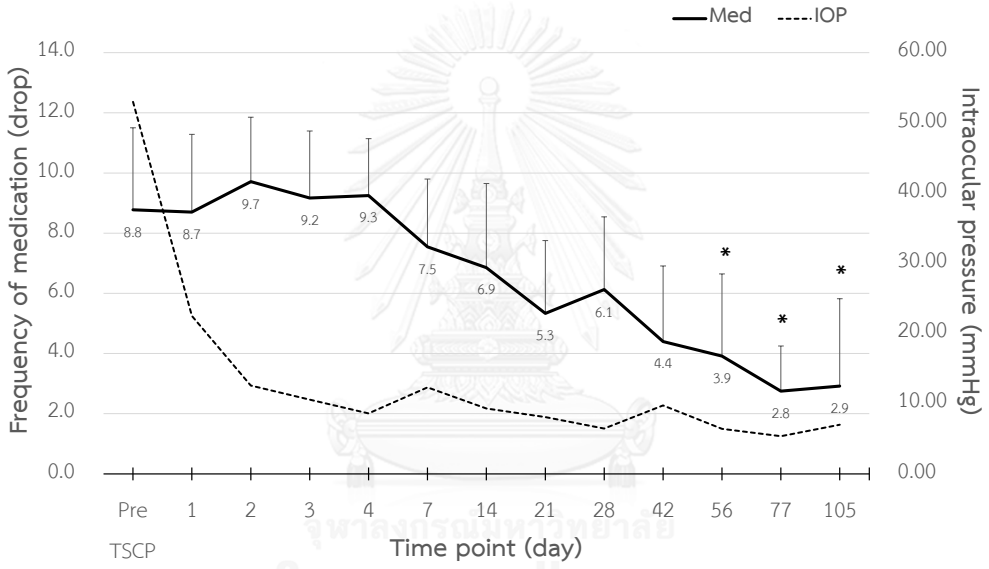

Figure 21 Mean number of topical hypotensive application (drops) per day ± SD at pre/post TSCP **protocol II**. Asterisk (*) indicates significantly difference of mean number of topical hypotensive application at *p<0.05*.

Protocol III (Figure 22)

Mean number of topical hypotensive applications at pre TSCP was 9.83±3.56 drops (7.57-12.10 95%CI), which was ranged from 4-16 drops per day. Following TSCP, mean number of eye drops was shortly maintained for 2 days, and then slowly decreased. It was slightly raised to 7.75±5.14 drops per day (5.01-10.49 95%CI) at day 28 post TSCP before markedly decreased throughout the rest of the study with statistical significance (*p<0.05*) since day 56 post TSCP. Mean number of hypotensive eye drops at day 105 post TSCP was 1.08±0.80 drops per day (0.24-1.92 95%CI). One out of ten eyes no longer required topical medications at the end of the study.

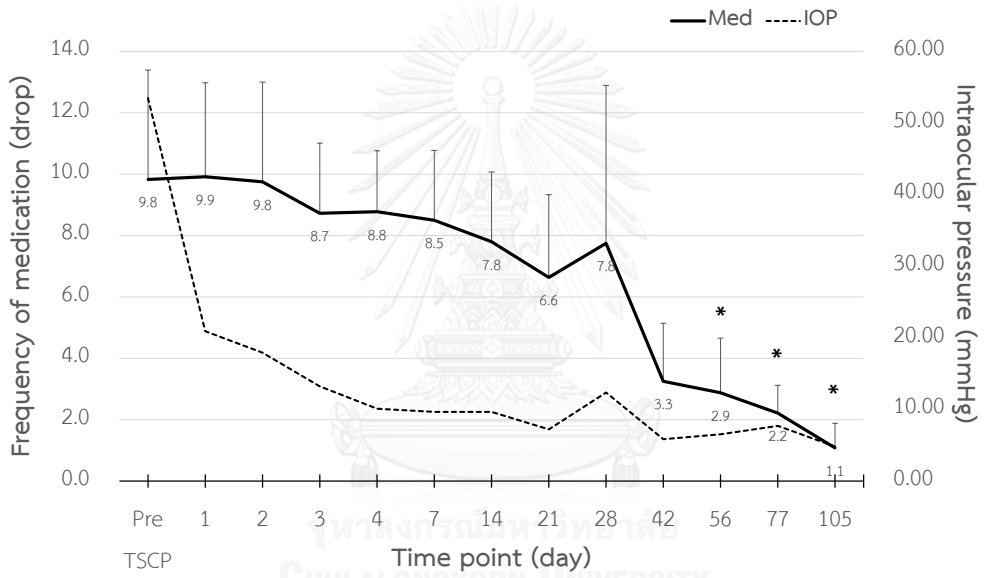

Figure 22 Mean number of topical hypotensive application (drops) per day ± SD at pre/post TSCP **protocol III**. Asterisk (*) indicates significantly difference of mean number of topical hypotensive application at *p<0.05*.

Comparison of the difference in number of topical hypotensive applications among three TSCP protocols revealed statistically significant difference (*p<0.05*) between protocol I and III on day 4 and 42 post TSCP.

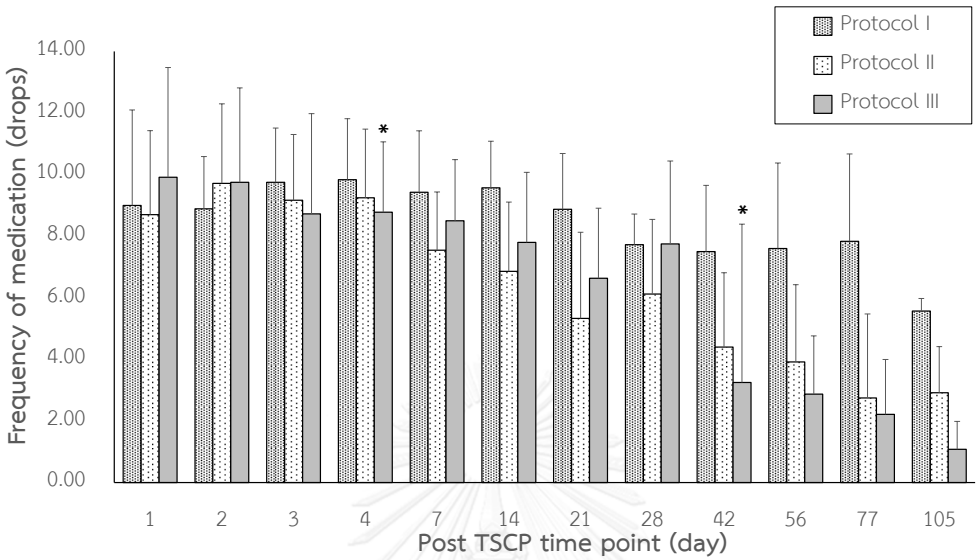

Figure 23 Mean number of topical hypotensive application (drops) per day ± SD from protocol I, II and III post TSCP. Asterisk (*) indicate significant difference of eye drop reduction between protocol I and III at *p<0.05*.

4. Axial Globe Length

Mean values of AGL at pre TSCP were 20.87±0.97, 20.17±1.33 and 21.14±1.87 mm in protocol I, II and III, respectively. Day 28 post TSCP, mean AGL of all groups decreased. The mean length was 20.47±0.75 and 19.75±0.92 mm in protocol I and II, respectively, whereas it obviously decreased to 17.80±0.85 mm in protocol III. It slightly increased on day 56 post TSCP only in protocol I though it continued to decrease and comparable in protocol II and III (17.30±2.55 and 17.50±1.27 mm). At day 105 post TSCP, mean AGL values were 18.98±2.00, 17.27±1.80 and 16.32±2.01 mm, in protocol I, II and III, respectively. Statistical analysis revealed significant reduction of the mean AGL difference (*p<0.05*) as compared to pre TSCP in protocol III at day 105 post TSCP.

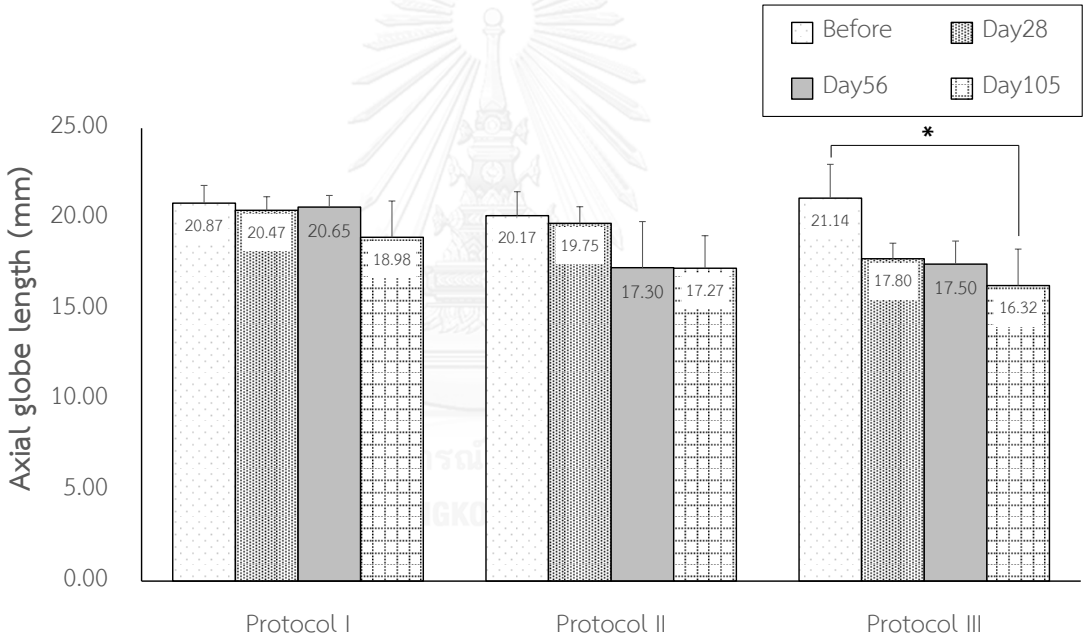

Figure 24 Mean axial globe length (mm) ± SD at pre/post TSCP, before TSCP and 28, 56 and 105 day after TSCP. Asterisk (*) indicates significantly difference of mean AGL reduction within group at *p<0.05*.

Different TSCP protocols provided various clinical results (Table 3). Protocol III had achieved the highest qualified success rate (100%) to lower the IOP below 21 mmHg by the end of the study. It was evident that protocol III provides the most reduction of IOP, frequency of topical hypotensive medication as well as AGL.

Protocol	**Success rate** (%)	**IOP reduction** (%)	**Medication reduction** (%)	**AGL reduction** (%)
I	60	73.34	41.98	9.05
II	90	86.36	66.74	14.31
III	100	91.18	89.01	22.80

Table 4 Summary of success rate, IOP reduction, topical medication reduction and AGL reduction (in percentage) of three different TSCP protocols.

5. Clinical complications

Ocular complications observed in this study included scleral perforation (Figure 25A), corneal ulcer, corneal edema, hyphema (Figure 25B), aqueous flare, hypotony and atrophic eye (Figure 25C).

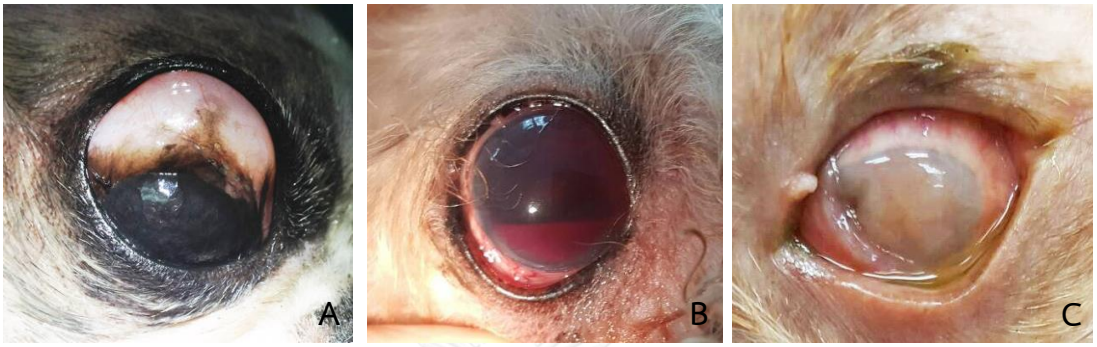

Figure 25 Clinical complications from TSCP procedure were noted. (A) scleral perforation (B) hyphema and (C) atrophic eye.

Protocol I

Scleral perforation was not evident during TSCP. Hyphema was observed in 1 eye (1/10; 10%) since day 1 after TSCP. Three eyes had aqueous flare (3/10; 30%). One eye was found at day 3 while the other two were found at day 14. The one that aqueous flare occurred at day 3 did not respond well to treatment. That eye shortly developed deep corneal ulcer and hypopyon. This eye was therefore enucleated. The other two eyes were from the same dog. Severe ocular pain was observed. These two eyes were as well then enucleated. Corneal edema suddenly appeared at day 3 after TSCP in 1 eye (1/10; 10%), then disappeared on the next day after treatment. Corneal ulcer is the most common complication in eyes receiving TSCP protocol I (5/10; 50%). Following TSCP, majority of eyes developed corneal ulcer during the first week; refractory corneal ulcer and small stromal corneal ulcer. After being treated, they recovered within 1-2 weeks. Another eye was the same that previously had aqueous flare and ended up with enucleation. Hypotony was noticed in 4 eyes (4/10; 40%) at median day 49.5 after TSCP. Out of hypotony group, one eye later had gone through atrophic eye (1/10; 10%).

Protocol II

Scleral perforation occurred in 3 eyes (3/10; 30%). It occurred at the dorsotemporal region of the eyeball. Sclera rapidly healed afterwards. Corneal edema was found in 2 eyes (2/10; 20%). One immediately developed after TSCP procedure while the other developed the following day after TSCP. Corneal edema of both eyes had soon resolved. Hyphema occurred in 3 eyes (3/10; 30%). One of these eyes also had aqueous flare (1/10; 10%) since day 1. This dog was not well taken care by the owner, who decided this dog to be terminated by enucleation. The other two eyes that hyphema were observed at day 20 and 22 post TSCP had resolved after medical treatment. Corneal ulcer developed in 6 eyes (6/10; 60%) during the first week after being treated. Most were stromal in depth while a few was complicated. Corneal ulcers were all treated except the one initially developing aqueous flare. Hypotony was noted in 4 eyes (4/10; 40%) at median day 45.5, one of which sooner was atrophic eye at day 46 (1/10; 10%).

Protocol III

Scleral perforation occurred in 4 eyes (4/13; 30.77%) mostly found on dorsotemporal part of the eye. Sclera rapidly healed. Corneal edema immediately occurred in two eyes, both of which had quickly resolved. Corneal edema of the other eyes developed at day 1 post TSCP, which disappeared soon after. Aqueous flare was noted in 2 eyes (2/13; 15.38%) at day 2 (both eyes from the same dog). It well responded after treatment of systemic corticosteroid. Hyphema developed in 3 eyes (3/13; 23.08%). All occurred during the first 3 days after TSCP. It quickly faded after medical treatment and restricted animal's activity. Corneal ulcer was the major complication in this protocol. It was found in 9 out of 13 eyes (9/13; 69.23%) at median day 5 (range 1-16 day post TSCP). There were 1 deep, 4 stromal and 4 complicated corneal ulcers. Ulcers were mostly located at center of the cornea. Eight ulcers were resolved after medical treatment, while the deep ulcer had additional supportive therapy by nictitating membrane flap. Hypotony was observed in 8 eyes (8/13; 61.54%)

at median day 56, six of which had turned atrophic eye (6/13; 46.15%) at median day 41.5.

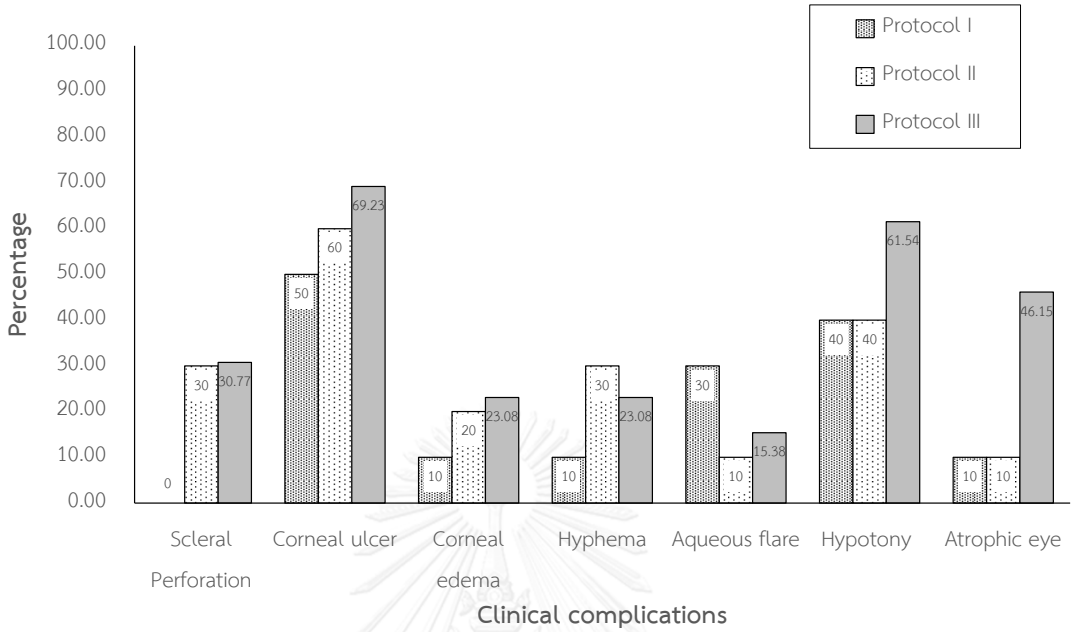

Figure 26 Ocular complications following TSCP in three different protocols.

6. Correlation study

Reduction of IOP, topical hypotensive medication and AGL

Difference of IOP, number of topical hypotensive medication and AGL between pre and post TSCP values of each eye was calculated, then compared with total amount of laser energy delivered per eye.

There was no correlation between total energy delivery per eye (X) and difference of IOP (Y) in the study: Y = 45.314+0.004X; correlation coefficient = 0.011, R^2=0.001, *p=0.95*. There was statistically significant association between total energy delivery per eye (X) and difference of topical hypotensive medication (Y): Y = -2.319+0.046X; correlation coefficient = 0.543, R^2=0.295, *p=0.001*. Total energy delivery per eye (X) and difference of axial globe length (Y) were related; regression formula: Y = 0.6+0.017X; correlation coefficient = 0.344, R^2=0.118, *p=0.068*.

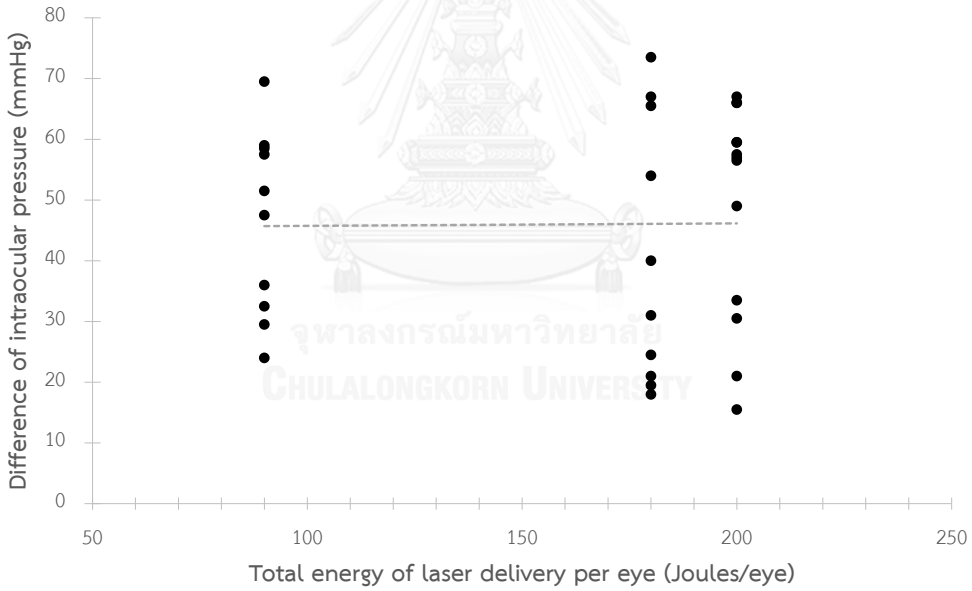

Figure 27 Regression and correlation between total energy delivery per eye (X) and difference of IOP (Y). Correlation not significant: correlation coefficient, 0.011; *p=0.95*; Y = 45.314+0.004X; R^2=0.001.

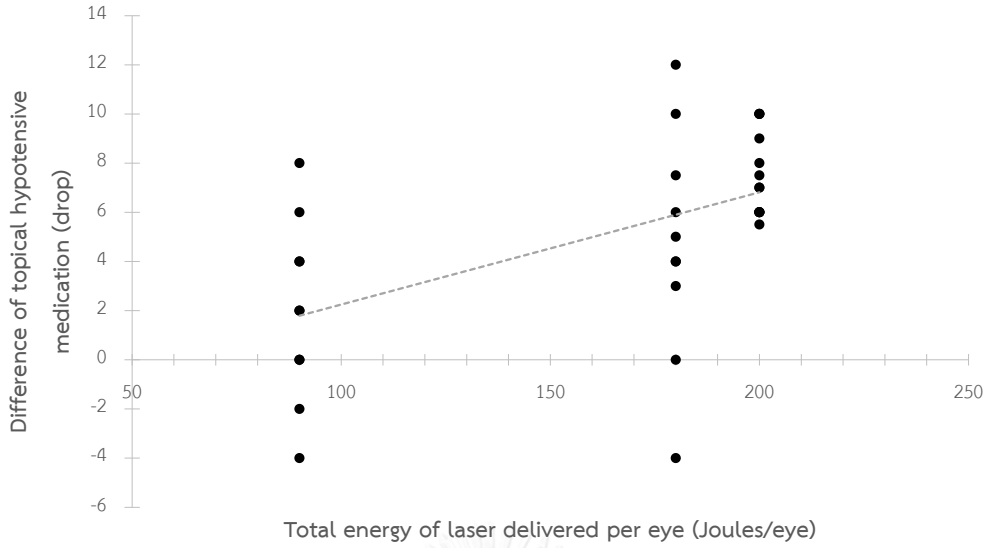

Figure 28 Regression and positive correlation between total energy delivery per eye (X) and difference of topical hypotensive medication (Y). Statistically significant correlation: correlation coefficient = 0.543; *p=0.001*; Y = -2.319+0.046X; R^2=0.295.

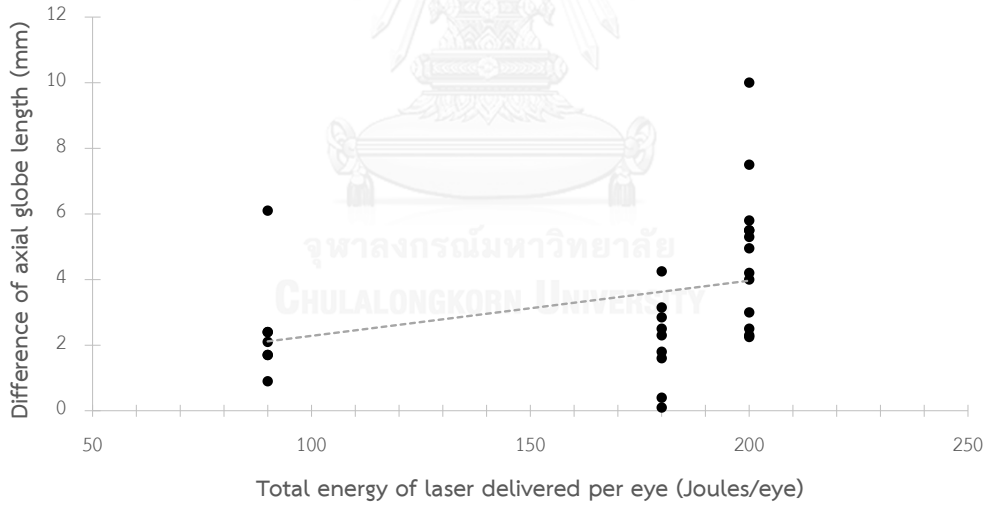

Figure 29 Regression and positive correlation between total energy delivery per eye (X) and difference of axial globe length (Y). Statistically significant association: correlation coefficient = 0.344; *p=0.068*; Y = 0.6+0.017X; R^2=0.118.

However, statistically significant association was found between percent reduction of IOP (Y) and baseline IOP (X) in this study: Y = 65.670+0.321X; correlation coefficient = 0.379, R^2=0.144, *p=0.029*.

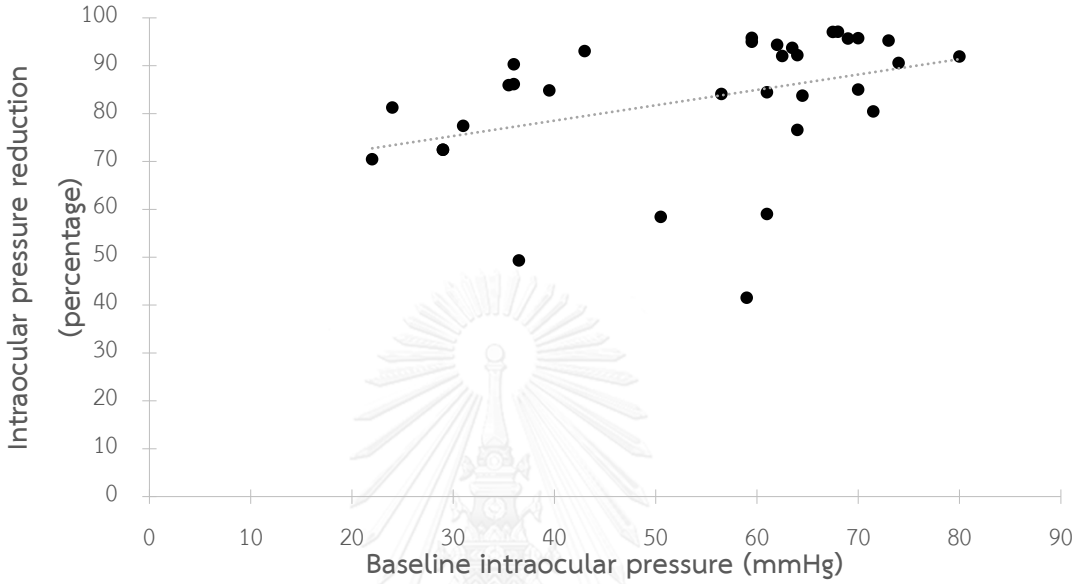

Figure 30 Regression and positive correlation between baseline IOP (X) and percent reduction of IOP (Y). Statistically significant association: correlation coefficient = 0.379; p=0.029; Y = 65.670+0.321X; R^2=0.144.

Audible "pop" sound

Statistically significant negative association was found between laser energy per laser application (X) and audible "pop" sound (Y): Y = 177.212-49.038X, correlation coefficient = 0.381, R^2=0.145, *p=0.034*.

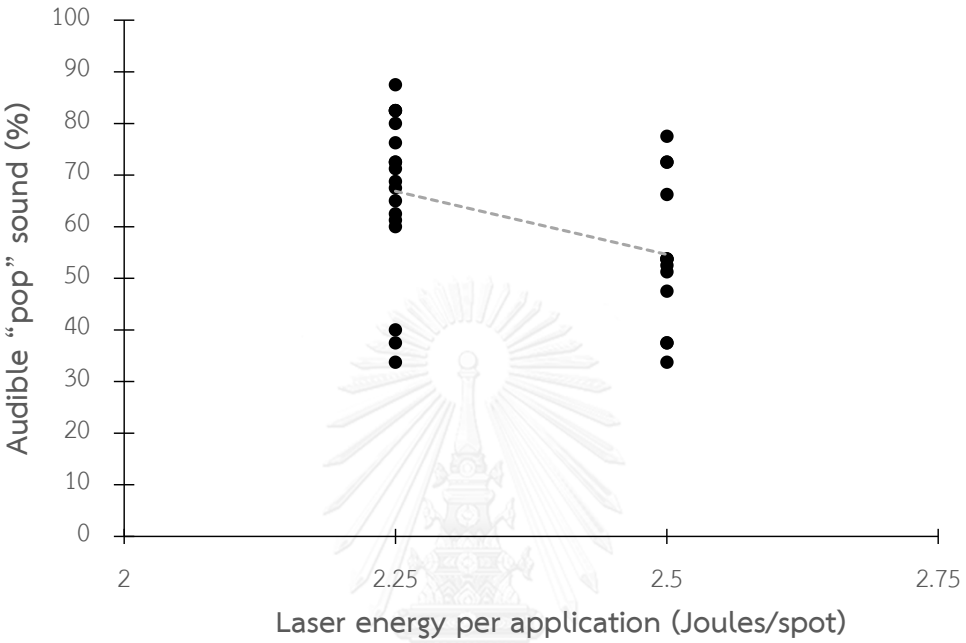

Figure 31 Regression and correlation between laser energy per application (X) and audible "pop" sound (Y). Statistical significant: correlation coefficient = 0.381; p=0.034; Y = 177.212-49.038X; R^2=0.145.

Ocular complications

Positive correlation with statistical significance was found between laser energy per application (X) and atrophic eye (Y): Y = -19.407+7.701X; correlation coefficient = 0.395, R^2=0.156, *p=0.039*.

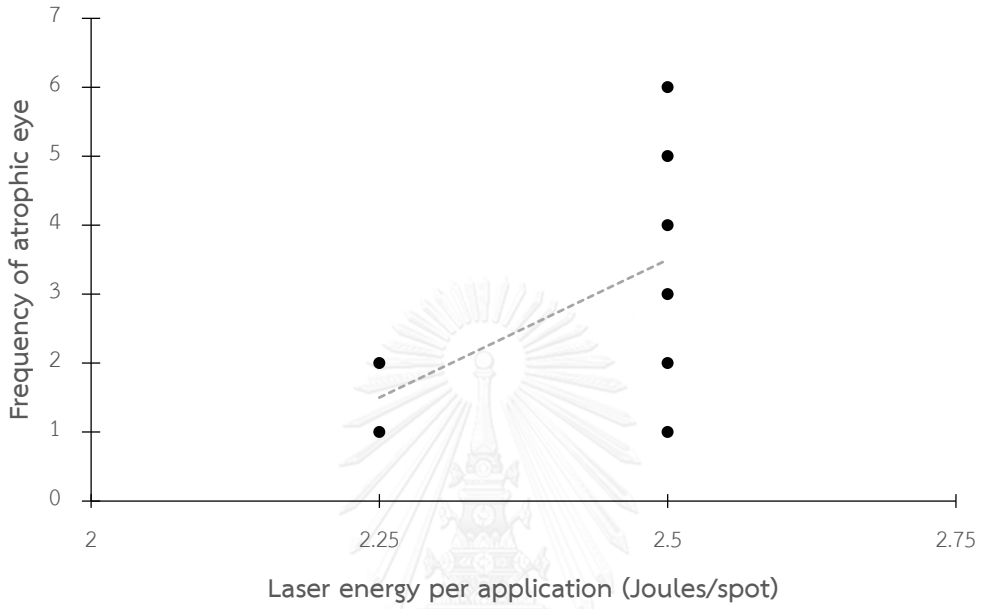

Figure 32 Regression and positive correlation between laser energy per application (X) and number of atrophic eye (Y). Statistically significant correlation: correlation coefficient = 0.359; p=0.039; Y = -19.407+7.701X; R^2=0.156.

Part 2: Study of pathological changes of ciliary body following different TSCP protocols.

Destruction of pars plicata was observed at laser site of application in all TSCP protocols (Figure 31 and 32). Bilayer ciliary epithelium was separated. Structure of ciliary process could no longer be identified due to serious cell damage in protocol I and II. Non-pigmented epithelium was markedly disrupted at an exact exposure area. Dispersion of melanin pigments was found and confirmed by Masson's Fontana staining (Figure 33C). In the adjacent area, vacuolations were formed in non-pigmented ciliary epithelium to the lessor extent of laser exposure.

Severe disruption was observed in ciliary stroma after receiving TSCP protocol I and II rather than protocol III. Obvious cell loss was noted (Figure 31 and 32). Ciliary muscle fibers were in disorganize manner. Coagulative necrosis was well appreciated in ciliary body as well as the sclera along laser-exposed region, which were stained with Masson's trichrome. Protein coagulation in affected collagen fibers within sclera was observed. Diffuse collagen damage in the adjacent area was demonstrated (Figure 33B).

Damage to pars plana region was not observed after TSCP in all protocols (Figure 35 and 36). However laser misdirection occurred in the eye receiving TSCP protocol I. coagulative streak from sclera through ciliary body was supposedly from laser exposure at the location of 5 mm posterior to the limbus. Separation was apparent between ciliary stroma and sclera. Vacuoles developed in the non-pigmented ciliary epithelium (Figure 34B).

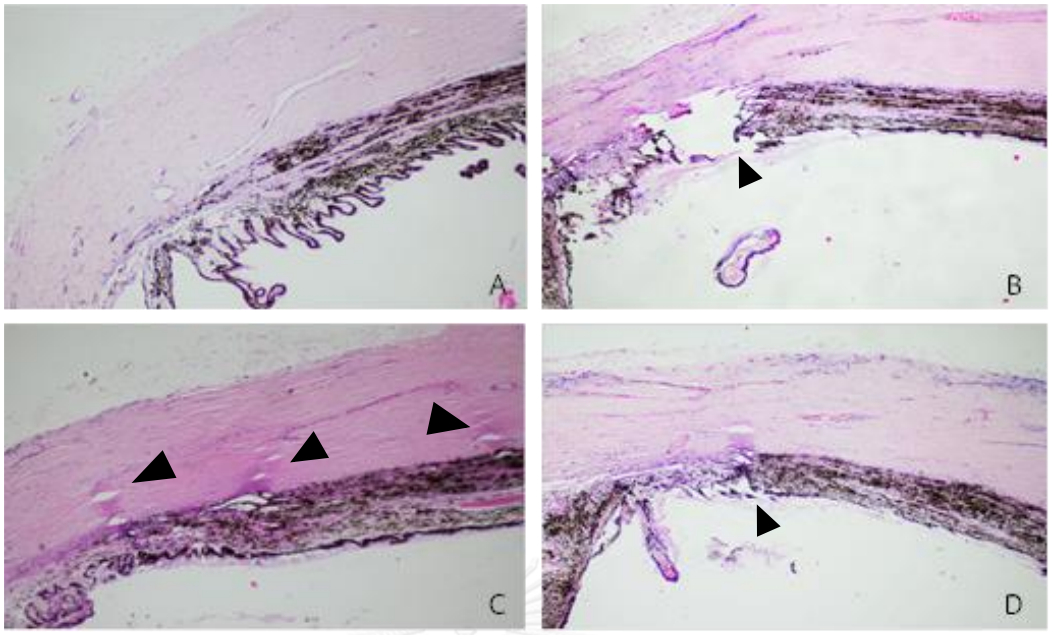

Figure 33 Photomicrograph of TSCP-treated pars plicata (H&E stain, original magnification x4)

(A) Non TSCP-treated glaucomatous eye. Narrow ciliary cleft and accumulation of pigments were noted. Contour of ciliary stroma, ciliary pigmented and non-pigmented epithelial cells were relatively normal.

(B) TSCP Protocol I-treated eye. Massive destruction of pars plicata and ciliary stroma was revealed. Marked precipitation of denatured protein was noticed within pars plicata and sclera along laser-exposed region. Dense basophilic staining represented coagulative necrosis (arrow, H&E stain).

(C) TSCP Protocol II-treated eye. Three areas of laser exposure were noted. Coagulative necrosis was present. Focal separations of ciliary stroma from sclera along laser-exposed regions occurred next to each other. Non-pigmented epithelium was disrupted at some extent, associated with vacuoles (arrows, H&E stain).

(D) TSCP Protocol III-treated eye. Disruption of pars plicata was observed. Some ciliary processes were intact (arrow, H&E stain).

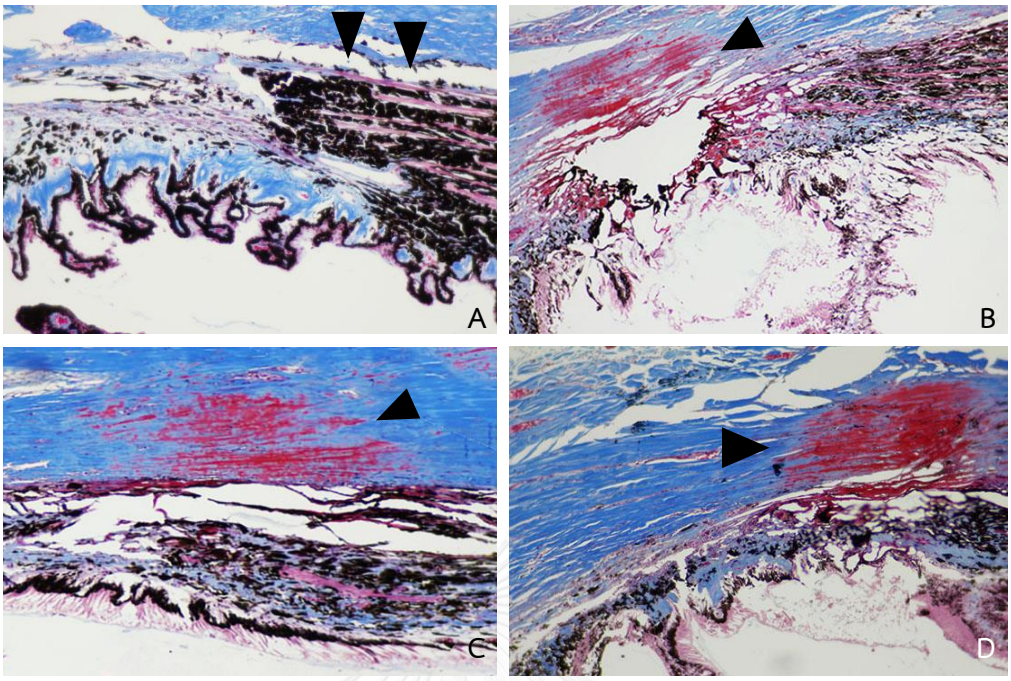

Figure 34 Photomicrograph of TSCP-treated pars plicata.

(A) Non TSCP-treated glaucomatous eye. MT stained non-pigmented epithelium and ciliary muscle in pink, collagen fibers in blue and nucleus in black (Masson's trichrome stain, original magnification x10).

(B) TSCP Protocol I-treated eye. Non-pigmented ciliary epithelium was apparently disrupted. Ciliary body, at which was directly exposed to laser showed marked damage with obvious cell loss. Ciliary muscles are in disorganized pattern. Coagulative necrosis of sclera was noted at laser-treated area (arrow).

(C) TSCP Protocol II-treated eye. Large separation between ciliary muscle and sclera; bilayer ciliary epithelium and ciliary muscle were observed. Non-pigmented epithelial cells were vacuolated (arrow).

(D) TSCP Protocol III-treated eye. Mild to moderate degree of tissue separation was noted. Ciliary epithelium was disrupted (arrow).

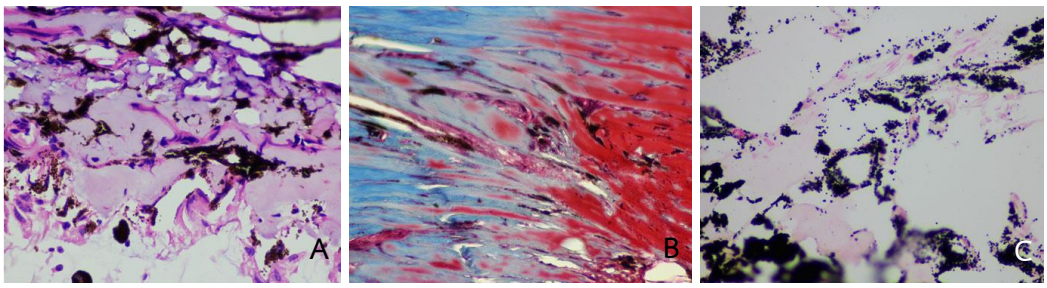

Figure 35 Photomicrograph of TSCP-treated pars plicata stained with special staining.

(A) Collagen necrosis in ciliary stroma (PAS stain, magnification x40).

(B) Diffuse collagen damage (red), normal collagen fibers (blue) in sclera (Masson's trichrome stain, magnification x40)

(C) Melanin pigment dispersion at ciliary epithelium (Masson's Fontana stain, magnification x40)

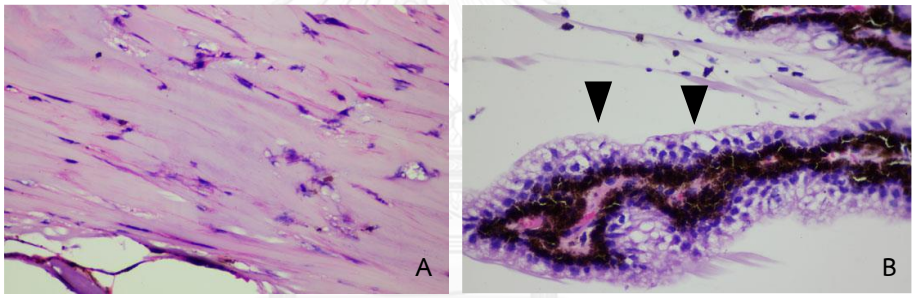

Figure 36 Photomicrograph of degenerative change in TSCP-treated eye. (H&E stain, original magnification x40)

(A) Protein coagulation of affected collagen fibers in sclera (H&E stain).

(B) Formation of vacuoles in non-pigmented ciliary epithelium (arrows, H&E stain)

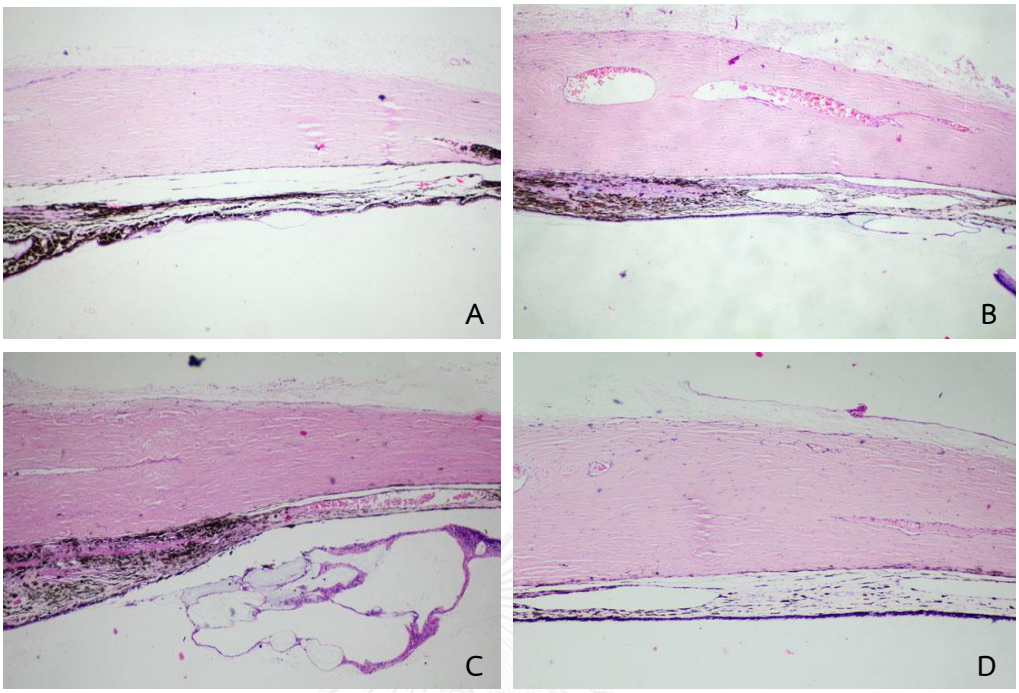

Figure 37 Photomicrograph of TSCP-treated pars plana stained with H&E. (Original magnification x4)

(A) Non TSCP-treated glaucomatous eye.

(B-D) TSCP-treated eye protocol I, II and III. Pathological changes were not observed. Tissue separation was from artifact.

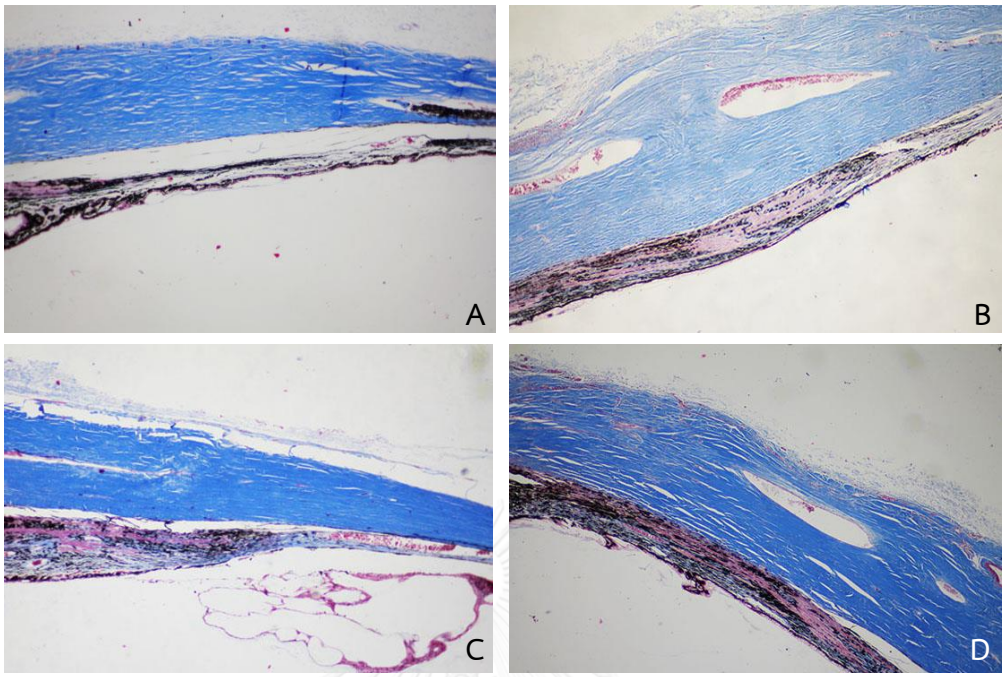

Figure 38 Photomicrograph of pars plana

(A) Non TSCP-treated glaucomatous eye (Masson's trichrome stain, original magnification x4)

(B-D) TSCP-treated eye protocol I, II and III. Pathological changes were not observed.

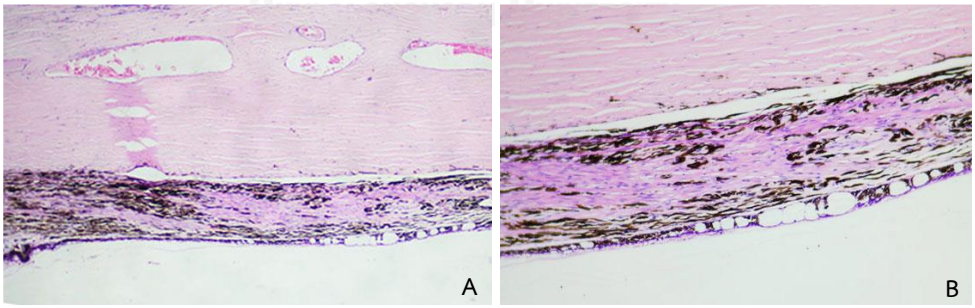

Figure 39 Photomicrograph of TSCP protocol I-exposed pars plana (H&E stain).

(A) Coagulative necrosis and tissue separation noted along laser-exposed region on the sclera. (Original magnification x4)

(B) Non-pigmented epithelium filled with vacuoles. (Original magnification x10)

CHAPTER 5

Discussion and Conclusion

TSCP has gained its popularity as a non-invasive, highly effective treatment for canine glaucoma nowadays (Hardman and Stanley, 2001). Many kind of lasers have been reported to use in veterinary ophthalmology, especially TSCP; such as, Nd:YAG (1064 nm) laser (Whigham et al., 1999) and diode laser (Hardman and Stanley, 2001). For diode laser, there are 2 different wavelengths using in ophthalmology, 532 and 810-nm, with different properties. 532-nm diode laser, blue-green laser, is better absorbed by melanin pigments, but low penetration of laser due to its short wavelength. Therefore, 810-nm diode laser, infrared laser, has better penetration property than that of 532-nm diode laser which is more suitable to use transsclerally as in TSCP (Alfred et al., 2013).

IOP is satisfactorily controlled under a defined level (21 mmHg) in eyes treated with TSCP protocol II and III, which are taken with the same number of laser spot, while total laser energy per eye is comparable; 180 and 200 Joules in protocol II and III, respectively. This is in an agreement with a report of Hauber and Scherer (2002) that total energy is significantly correlated to the success rate. In terms of IOP reduction, even though it rapidly reduces at comparable percentage between protocol I and II, success rate of protocol I is lower. Mean reduction of IOP is strongly related to total number of laser spot (Tzamalis et al., 2010). We therefore speculate that the less success rate in eyes receiving TSCP protocol I is possibly due to inadequate number of laser application, generating insufficient area of coagulative necrosis. Marked localized area of pars plicata disruption does not prove that adequate decrease of aqueous humor production occurs. The least reduction of the mean AGL in TSCP protocol I-treated eyes is as a result. For those TSCP protocol I-treated eyes that ended up with serious complications and enucleation, IOPs are extremely high and uncontrollable. On the other hand, less degree of ciliary disruption and cell separation

observed in protocol II and III may imply satisfactory summation of laser energy to destroy non-pigmented ciliary epithelium.

With the use of three TSCP protocols designed in this study, hypotensive drug application is reduced with the greatest percentage observed in protocol III. Reduction of hypotensive medication can be achieved when IOP is under control (Shahid, 2013) Not only a decrease of topical application helps to avoid ocular and systemic side effect of these medications, but it avoids vital economic issue. Statistical significant difference of drug application between protocol I and III, observed toward the end of the study, implies less effectiveness of aqueous humor producing cells destruction by diode laser in protocol I.

Rapid reduction of IOP of eyes treated with TSCP protocol III, on the other hand, demonstrates utmost atrophic eye and hypotony. Decrease of AGL was noted in all protocols with moderate degree of correlation. Only atrophic eye has statistically significant correlation to total energy of laser delivery per eye. While Spencer and Vernon (1999) reported hypotony as a direct proportion to total dosage of laser energy delivery per eye. Increase occurrence of hypotony is evident in human with neovascular glaucoma (Ramli et al., 2012).

Micro explosion of cells receiving extremely high laser energy results in “pop” sound during TSCP procedure. Percentage of “pop” sound is not correlated to the success in lowering IOP (Rebolleda et al., 1999), similar to our study. High percentage of “pop” sound however is related to greater severity of post TSCP anterior uveitis (Rebolleda et al., 1999). As noticed in protocol I, aqueous flare exceedingly occurs and proceeds to enucleation. Aqueous flare is a result of blood-ocular barrier disruption due to the vascular effect of congestion and mural necrosis (Birngruber, 1984). Similarly, Cook C (1997) report the presence of aqueous flare in most case after performed diode laser TSCP in canine glaucoma.

Clinical complications are comparable among three TSCP protocols. They are categorized into 2 groups depend on the time of detection; early and late phase. Incidence of corneal damage and anterior uveitis is noted as early complication, while atrophic eye and hypotony occur at late onset. Major clinical complication of early phase complication is corneal ulcer, which may be in accordance with thermal damage

of diode laser causing desensitization of corneal innervation (Johnson, 1998). Temporary tarsorrhaphy, a method to protect corneal surface can decrease occurrence of corneal ulcer (Hardman and Stanley, 2001).

Intraoperative scleral perforation is rare in human practice (Schlote, 2008). It is usually observed in preoperative scleral thinning eyes. As in our study, majority of scleral perforation occurs at scleral thinning region. However, incidence is apparent in eyes which are treated with TSCP protocol II and III. It is suggested that zone of thermal destruction is greater as compared to protocol I. This zone of tissue damage may be created by rise of tissue temperature from heat accumulation in the surrounding tissue (Niemz, 2013). Although destruction of sclera along laser exposure region receiving TSCP protocol I histologically seems serious from Masson's trichrome staining, accumulation of heat is still insufficient to create tissue perforation.

Histological comparison of pars plicata among three different laser protocols reveals different degree of degenerative coagulation. Excessive disruption is majorly found in TSCP protocol II-treated eyes, of which twice laser applications are subsequent to each other. Therefore frequency of laser application per quadrant has reached the highest among three TSCP protocols. As a result, marked cell disruption leads to severe post TSCP inflammation and hyphema. It is interesting that incidence of hyphema is noted in post TSCP eyes receiving protocol II and III. Hyphema develops at late phase after TSCP. It is suggested that preiridal fibrovascular membrane is formed due to severe inflammation (Peiffer et al., 1990). Bleeding can easily occur from newly-formed blood vessels. In contrary, hyphema develops within 3 days after TSCP protocol III may relate to hemorrhage from uveal blood vessels congestion.

Slow coagulation procedure, comprised of low laser power accompanying with long laser exposure time, as in protocol III has become more popular in human glaucoma with dark iris (Kaushik et al., 2008). Slow coagulation TSCP still delivers significant amount of energy to the ciliary body epithelium, while reducing complications of dyscoria, hyphema, and phthisis bulbi as compared to other cyclodestructive technique (Hardman and Stanley, 2001).

From being exposed to laser at pars plicata, pathological changes are not observed at pars plana. Diode is laser results in reduced collateral tissue effects

(Hardman and Stanley, 2001). Separation of ciliary stroma from sclera observed in this study is suggested from tissue processing rather TSCP. Observation of coagulative necrosis is found at the anterior part of pars plana in protocol II-treated eye. This may be related to laser exposure at 5 mm distance posterior to the limbus in relatively non-buphthalmic eyeball. Application of laser at this distance is therefore recommended in enlarged eyeballs.

Advantages of the study

A study of three different protocols comparison has provided useful information and new knowledges about laser setting as a predictor for optimal TSCP protocol, which is the goal of this study. Even a suitable TSCP protocol for individual is still unpredictable, but more confidentially understandable in TSCP performance is gained from knowledge obtaining from this study.

Limitation of the study

TSCP parameters other than the study parameter should be controlled in different setting of TSCP protocols in order to exactly define which parameter has influence to the clinical outcomes.

Conclusion

Three different transscleral diode laser cyclophotocoagulation protocols demonstrates different success rate greatly dependent on the number of the laser application and total laser energy delivery per eye. Degree of pathological injury is related to laser transmission from sclera to aqueous producing cells. To reduce post TSCP complications, photodisruption is avoided by reducing laser energy per spot.

Suggestion

Among three different TSCP protocols, protocol III seems to have the most desirable result in IOP control, decrease of topical hypotensive medication use and minimize the size of eyeball. Although more ocular complications occurred, but they were treatable complications, except for an atrophic eye which is not serious complication in veterinary field. Further study of slow coagulation TSCP protocols is suggested in canine chronic glaucoma. Number of laser exposure application should be reduced to lower clinical complications.

Anatomical study of ciliary body position in different AGL of the glaucoma eye and their correlation would provide many advantages in appropriately selection of laser probe placement position to avoid over-destruction of the ciliary body.

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APPENDIX

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