การใช้เลเซอร์ชนิดไดโอดผ่านเปลือกหุ้มลูกตาเพื่อรักษาต้อหินระยะสุดท้ายในสุนัข

นางสาวภัทรพร วินะยานุวัติคุณ

วิทยานิพนธ์นี้เป็นส่วนหนึ่งของการศึกษาตามหลักสูตรปริญญาวิทยาศาสตรมหาบัณฑิต
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TRANSSCLERAL DIODE LASER CYCLOPHOTOCOAGULATION FOR THE TREATMENT OF ABSOLUTE GLAUCOMA IN DOGS

Miss Pataraporn Winayanuwattikun

A Thesis Submitted in Partial Fulfillment of the Requirements
for the Degree of Master of Science Program in Veterinary Surgery
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Thesis Title	TRANSSCLERAL DIODE LASER CYCLOPHOTOCOAGULATION FOR THE TREATMENT OF ABSOLUTE GLAUCOMA IN DOGS
By	Miss Pataraporn Winayanuwattikun
Field of Study	Veterinary Surgery
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ภัทรพร วินะยานุวัติคุณ : การใช้เลเซอร์ชนิดไดโอดผ่านเปลือกหุ้มลูกตาเพื่อรักษาต้อหินระยะสุดท้ายในสุนัข (TRANSSCLERAL DIODE LASER CYCLOPHOTOCOAGULATION FOR THE TREATMENT OF ABSOLUTE GLAUCOMA IN DOGS) อ.ที่ปรึกษาวิทยานิพนธ์หลัก: ผศ. สพ.ญ. ดร.นลินี ตันติวนิช, 60 หน้า.

การใช้เลเซอร์ชนิดไดโอดในการรักษาต้อหินระยะสุดท้ายเป็นการฉายแสงเลเซอร์ผ่านเปลือกหุ้มลูกตาเพื่อทำลายเยื่อบุผิวของซิลิอารีบอดี โดยทำให้แขนงซิลิอารีเกิดการตายแบบจับตัวเป็นก้อนโดยที่ไม่ต้องผ่าตัดเข้าไปในลูกตา ทำให้มีการสร้างของเหลวในลูกตาน้อยลง งานวิจัยนี้ศึกษาผลของการใช้วิธีดังกล่าวในสุนัขต้อหินระยะสุดท้าย จากการศึกษาลักษณะทางกายวิภาคของซิลิอารีบอดีในตาสุนัขที่โปนจากภาวะต้อหิน จำนวน 12 ตา พบว่าระยะทางจากขอบกระจกตาถึงขอบหลังสุดของซิลิอารีบอดีส่วนหน้า มีค่าเฉลี่ยตั้งแต่ 4.44 ± 0.74 ถึง 6.11 ± 0.73 มิลลิเมตร การฉายแสงเลเซอร์ชนิดไดโอดผ่านเปลือกหุ้มลูกตาในตาที่เป็นต้อหินระยะสุดท้าย จำนวน 31 ตา จากสุนัข 26 ตัว โดยใช้กำลังของแสงของเลเซอร์ที่ 1500 มิลลิวัตต์ และระยะเวลาที่เนื้อเยื่อได้รับแสง 1500 มิลลิวินาที ในการยิงเลเซอร์ผ่านเปลือกหุ้มลูกตา 360 องศา รอบลูกตาที่ตำแหน่งห่างจากขอบกระจกตา 3, 4 และ 5 มิลลิเมตร เป็นจำนวนทั้งสิ้น 64 ตำแหน่ง พบว่าร้อยละของจำนวนครั้งที่ได้ยินเสียง “ป๊อป” เท่ากับ 71.97 ค่าเฉลี่ยของความดันในลูกตาลดลงจาก 62.1 ± 2.5 มิลลิเมตรปรอทก่อนฉายแสงเลเซอร์เหลือ 6.6 ± 1 มิลลิเมตรปรอทในวันสุดท้ายของการศึกษา ความยาวตามแนวแกนของลูกตาลดลงอย่างมีนัยสำคัญและเทียบเท่ากับความยาวตามแนวแกนของลูกตาปกติ จำนวนครั้งของการใช้ยาหยอดเพื่อลดความดันในลูกตาลดลงอย่างมีนัยสำคัญตั้งแต่วันที่ 21 หลังการยิงเลเซอร์ และลดลงอย่างต่อเนื่องจนสิ้นสุดการศึกษา ภาวะความดันในลูกตาต่ำกว่าปกติเป็นภาวะแทรกซ้อนที่พบมากที่สุดในการศึกษานี้ รองลงมาคือ แผลที่กระจกตาชั้นตื้นและเลือดออกในช่องหน้าม่านตา จากการศึกษาลักษณะทางพยาธิวิทยาในตาสุนัขที่เป็นต้อหินระยะสุดท้าย จำนวน 6 ตา ที่ได้รับเลเซอร์ชนิดไดโอดผ่านเปลือกหุ้มลูกตา พบการตายแบบจับตัวเป็นก้อนที่ซิลิอารีบอดี เนื้อเยื่อที่ได้รับความร้อนมากเกินไปจะมีการแยกชั้นหรือการระเบิดของเนื้อเยื่อที่บริเวณดังกล่าว เกณฑ์วิธีการใช้เลเซอร์ชนิดไดโอดผ่านเปลือกหุ้มลูกตาของงานวิจัยนี้เหมาะสมสำหรับรักษาสุนัขต้อหินระยะสุดท้าย โดยประสบความสำเร็จในการควบคุมความดันในลูกตาให้ต่ำกว่า 20 มิลลิเมตรปรอท คิดเป็นร้อยละ 96.77 และภาวะแทรกซ้อนที่พบสามารถควบคุมได้

ภาควิชา ศัลยศาสตร์ ลายมือชื่อนิสิต .....

สาขาวิชา ศัลยศาสตร์ทางสัตวแพทย์ ลายมือชื่อ อ.ที่ปรึกษาหลัก .....

ปีการศึกษา 2557

5575314331 : MAJOR VETERINARY SURGERY
KEYWORDS: ABSOLUTE GLAUCOMA / CANINE / TRANSSCLERAL CYCLOPHOTOCOAGULATION / SEMICONDUCTOR DIODE LASER

PATARAPORN WINAYANUWATTIKUN: TRANSSCLERAL DIODE LASER CYCLOPHOTOCOAGULATION FOR THE TREATMENT OF ABSOLUTE GLAUCOMA IN DOGS. ADVISOR: ASST. PROF. NALINEE TUNTIVANICH, D.V.M., Ph.D., 60 pp.

Diode laser transscleral cyclophotocoagulation (TSCP) for the treatment of absolute glaucoma, is a noninvasive procedure which induces coagulation necrosis to the ciliary epithelium by laser light to reduce aqueous humor production. This study was to assess outcomes using TSCP in dogs with absolute glaucoma. Location of pars plicata in 12 buphthalmic canine globes was anatomically studied. Mean distance from limbus to posterior border of pars plicata was ranged from 4.44 ± 0.74 to 6.11 ± 0.73 mm. Diode laser TSCP at a setting of 1500mW power and 1500ms duration was performed on 31 eyes of 26 dogs diagnosed with absolute glaucoma. Laser probe was applied in 64 spots, 360 degrees around the globe at 3, 4 and 5 mm distance posterior to the limbus. 71.97% of 'pop' sounds were observed. Mean IOP significantly reduced from 62.1 ± 2.5 to 6.6 ± 1.0 mmHg by the end of the study. Axial globe length was significantly decreased, comparable to that of normal eyes. Significant reduction in numbers of topical hypotensive application was first observed at day 21 post TSCP. Hypotony was the most frequent complication, followed by superficial corneal ulcer and hyphema. Six eyes that were histologically examined revealed coagulative necrosis of ciliary body. Tissue separation and disruption were demonstrated if overheated. This laser protocol was suitable as a treatment for canine absolute glaucoma. Qualified success in accordance with IOP level of less than 20 mmHg was 96.77% and clinical complications were controllable.

Department: Veterinary Surgery Student's Signature .....
Field of Study: Veterinary Surgery Advisor's Signature .....
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LIST OF ABBREVIATIONS

%	=	percentage
ºC	=	degree celcius
ALP	=	alkaline phosphatase
ALT	=	alanine transaminase
BUN	=	blood urea nitrogen
Ca^{2+}	=	calcium
CAI	=	carbonic anhydrase inhibitor
Cl^-	=	chloride
CPC	=	cyclophotocoagulation
ECP	=	endolaser cyclophotocoagulation
e.g.	=	*exempli gratia* (for example)
etc.	=	*et cetera* (and the rest)
H	=	hour
HCO_3^-	=	bicarbonate
H&E	=	hematoxillin and eosin
IO	=	increase aqueous humor outflow
IOP	=	intraocular pressure
K^+	=	potassium
kg	=	kilogram
Mg^{2+}	=	magnesium
mg	=	milligram
ml	=	milliter
mm	=	millimeter
mmHg	=	millimeter of mercury
ms	=	millisecond
mW	=	milliwatt
Na^+	=	sodium
Nd:YAG	=	neodymium-doped yttrium aluminium garnet ($Nd:Y_3Al_5O_{12}$)

nm	=	nanometer
PACG	=	primary open angle glaucoma
PBS	=	phosphate buffer saline
POAG	=	primary angle closure glaucoma
SD	=	standard deviation
SE	=	standard error
SEM	=	standard error of mean
STT	=	schirmer tear test
TSCP	=	transscleral cyclophotocoagulation
µg	=	microgram
µm	=	micrometer

Chapter 1
Introduction

Importance and Rationale

Glaucoma is one of the major causes of global blindness. It is the disease characterized by elevated intraocular pressure (IOP), leading to degenerative changes of the optic disc and the retina. Numbers of glaucoma patients had been estimated to be 60.5 million people in 2010 (Quigley and Broman, 2006), and would possibly increase to 76.0 million in 2020 and 111.8 million in 2040 (Tham et al., 2014).

Pathogenesis of primary open angle glaucoma (POAG) in humans slowly develops. Therefore, ocular pain and blindness could be prolonged. In contrary, most canine glaucoma was classified as angle closure (Gelatt et al., 2007). Thus, IOP suddenly increases, leading to acute, nonreversible blindness and extreme ocular pain (Miller, 2008). As a result, glaucoma is the most common indication of enucleation in dogs (Steinmetz and Oechtering, 2008). Prevalence of primary and secondary glaucoma in dogs presented to veterinary teaching hospitals in North America has gradually increased (Gelatt and MacKay, 2004a; Gelatt and MacKay, 2004b) similar to that reported in humans.

When acute glaucoma progresses to chronic stage, other ocular complications would be associated with persistent elevated IOP. Animals are nonresponsive to medical treatment and permanently blinded. Advanced stage of glaucoma, to which eyeball is buphthalmic, is known as absolute or end-stage glaucoma. This stage of the disease is usually seen in dogs without early detection, correct diagnosis or rapid control of IOP elevation (Lin et al., 2007). Treatment would then be concentrated on relief of ocular pain and its complications. Long term use of topical glaucoma medications could cause ocular surface toxicity (Ammar et al., 2010). Cost of hypotensive drugs is extremely high while treatment is lifelong (Fiscella et al., 2003). As a result, surgery has become a more promising procedure for long-term treatment of canine glaucoma.

Diode laser cyclophotocoagulation (CPC) has gradually become more universal in human medicine for cyclodestruction. Laser energy is absorbed by pigmented ciliary epithelial cells, prior to being transformed into heat energy. With the rise of tissue temperature, target cells are destroyed via coagulative necrosis. Laser can be delivered through sclera (transscleral cyclophotocoagulation; TSCP) or directly to ciliary epithelial cells (endolaser cyclophotocoagulation; ECP). TSCP is a non-invasive, uncomplicated technique that surgical incision is not required (Lin, 2008). It had high success rate to lower IOP in various types of human glaucoma (Kramp et al., 2002). Not only it could be used in visual eyes to maintain vision, but also in non-visual eyes to cosmetically preserve eyeballs (Hardman and Stanley, 2001).

To achieve success in permanently lowering the IOP, several factors should have been considered (Hauber and Scherer, 2002). While prevalence of canine chronic glaucoma is increasing nowadays, clinical experiences of TSCP in veterinary practice are rather limited. To date, there has not yet been a report applying diode laser TSCP in dogs with absolute glaucoma. The aim of this study was to evaluate clinical outcomes and histological changes of target tissue with the application of diode laser TSCP at three different locations posterior to the limbus, 360 degree circumference, based on structural alterations of ciliary process in canine buphthalmic eyes.

Research questions

Primary research questions

1. Is pars plicata in buphthalmic globe unequally circumferential elongated?
2. Can laser setting protocol, which is used in this study permanently lower the IOP to the level of satisfaction?

Secondary research questions

1. What are clinical complications following TSCP?
2. What are pathological changes observed in canine buphthalmic eye following TSCP?

Objectives of the study

1. To study the location of pars plicata in buphthalmic canine eye.
2. To study clinical outcomes and complications after TSCP with the specific laser setting protocol in canine absolute glaucoma.
3. To evaluate the pathological change of TSCP on ciliary process and adjacent tissues with the specific laser setting protocol in canine absolute glaucoma.

Advantage of the study

TSCP is an alternative treatment for glaucoma in animals. Not only this laser setting protocol could be modified to apply in different types of glaucoma in various species, it could be used in ocular hypertension to preserve vision.

Chapter 2

Literature Review

Glaucoma is a common ophthalmic disease causing blindness in humans and animals. Obstruction in aqueous humor outflow is the initial cause of elevated IOP, which is too high to maintain normal optic nerve axoplasmic flow and blood flow. Visual-field loss and blindness are therefore results from retinal dysfunction and optic nerve degeneration.

Aqueous humor and its dynamic

Aqueous humor is a transparent fluid in the anterior and posterior chambers of the eye. It consists of numerous components, all of which are essential to supply nutrients and oxygen to ocular avascular tissues; cornea, lens, anterior vitreous. Its compositions are a variety of ions (Na^+, K^+, Ca^{2+}, Mg^{2+}, Cl^-, HCO_3^-), glucose, ascorbate, lactate, immunoglobulin, proteins, etc. (To et al., 2002) Not only has aqueous humor maintained optimal pressure in the globe, it has removed metabolic wastes of ocular tissue.

Aqueous humor is produced by the bilayered cilliary epithelium, which consists of pigmented and non-pigmented epithelium. It is secreted by three mechanisms; ultrafiltration, diffusion, and active transport. It flows from posterior chamber through the pupil into the anterior chamber, iridocorneal angle (figure 1) and then drains out of the eye via two outflow pathways (Goel et al., 2010). The main outflow pathway (conventional route) is via corneosclera. It passes through pectinate ligament, trabecular meshwork, angular aqueous plexus and finally to scleral venous plexus. The unconventional route is uveoscleral pathway, through which aqueous humor flows into suprachoroidal space. In dogs, most aqueous humor exits the eye through corneosclera; leaving 10% to 15% of it through uveosclera (Reinstein et al., 2009b). Balance of aqueous humor production and drainage is responsible for maintaining normal pressure inside the eye.

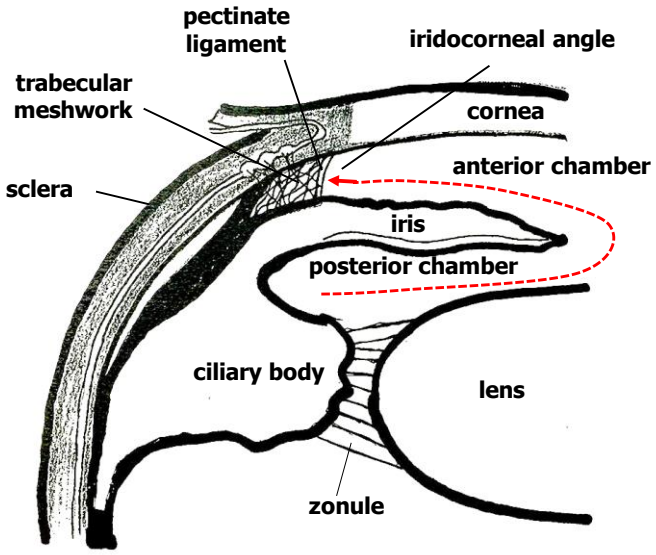

Figure 1. Schematic diagram demonstrating aqueous humor drainage pathway

Classification of the canine glaucoma

Primary glaucoma is a hereditary disease affected by malformation of iridocorneal angle at birth. Clinical signs would have not yet developed until dogs are 2-3 years of age or older (Gelatt and MacKay, 2004a). With this type of glaucoma, both eyes are rarely affected at the same time. The disease usually occurs in one eye, followed by the second eye in 40% of cases within two years (Strom et al., 2011a). By anatomical feature, two subtypes are characterized; primary open angle glaucoma (POAG) and primary angle closure glaucoma (PACG). Appearance of iridocorneal angle can be evaluated by the gonioscopy (Abrams, 2001).

POAG is the most common type of glaucoma in humans (Lee and Higginbotham, 2005). It was reported in certain breeds of dogs, which were beagles and Norwegian Elkhounds (Renwick, 2002). Angle is anatomically opened. IOP tends to slowly increase. Precise mechanism of POAG in dogs is unclear. However biochemical alteration in the trabecular meshwork is the most likely underlying cause resulting in greater resistance to aqueous outflow (Infeld and O'Shea, 1998). In beagles, POAG was

considered to be inherited in an autosomal recessive fashion (Miller, 2008), involving glycosaminoglycan accumulation in trabecular meshwork.

PACG is a major cause of blindness with high prevalence in Asian populations (Tham et al., 2014). It was reported in English Cocker Spaniel, American Cocker Spaniel, Basset Hound, Labrador Retriever, Golden Retriever and Siberian Husky (Renwick, 2002). Pathogenesis of PACG in dogs has not yet been revealed, but it is associated with goniodysgenesis (Renwick, 2002). As the IOP rises quickly, dogs have acute ocular pain, red eye and sudden blindness (Infeld and O'Shea, 1998). Immediate treatment to lower the IOP is necessary to relieve symptoms and prevent permanent loss of vision.

Secondary glaucoma is the other type of glaucoma encountered with other ocular abnormalities; such as uveitis, lens displacement, late-stage cataract, intraocular hemorrhage, ocular neoplasia, retinal detachment and trauma (Strom et al., 2011b). Although secondary glaucoma is not originally heritable, some of inciting causes (e.g. cataract, lens luxation) have genetic basis.

Ophthalmic signs

Severity of ophthalmic signs in canine glaucoma depends on IOP level, duration of the disease, underlying cause and age of the animal (Renwick, 2002).

Acute glaucoma occurs within a matter of hours with sudden high elevation of IOP (Lee and Higginbotham, 2005). Globe remains normal-sized with other ocular abnormalities; ocular pain, corneal edema, deep corneal vascularization, episcleral congestion and sudden loss of vision. By digital palpation, the affected eye is more rigid compared to normal eye. Some dogs develop other signs related to ocular pain; such as rubbing on the affected eye, depression, timidity, or aggressiveness (Miller, 2008). In humans, nausea and vomiting could be associated if ocular pain is intense (Lee and Higginbotham, 2005). Peripheral loss of vision initially developed (figure 2). Although complete vision loss can occur within a short period of time, optic nerve head damage and retinal ganglion cell dysfunction are potentially reversible, if IOP could immediately be controlled (Renwick, 2002).

Chronic glaucoma may arise as a consequence of unsuccessful control IOP or misdiagnosis of acute glaucoma. Although ocular pain is less as compared to acute type, it persists and prolongs with increasing chronicity. As a result, stretching of the cornea and sclera, as well as enlargement of the globe (buphthalmos) becomes prominent. Buphthalmos is irreversible even if pressure inside the eye is reduced afterward (Miller, 2008). Dogs are unable to completely close palpebral fissure due to enlarged eyeball. As the cornea stretches, linear ruptures in Descemet's membrane; so called descemet's streaks (Haab's striae), may occur, in addition to other corneal lesions. Optic disc cupping, optic disc atrophy and retinal degeneration were also found by direct or indirect ophthalmoscopic examination (Renwick, 2002). If glaucoma has approached to the end stage, lagophthalmos inducing exposure keratitis may occur, in association with other abnormalities such as recurrent corneal ulcer and lens luxation.

Figure 2. Change of visual field caused by glaucoma
A) Normal visual field; B) Peripheral visual field loss; C) Complete visual field loss.

Diagnosis

Precise diagnosis of glaucoma could be performed according to history, clinical presentation, and ophthalmic examinations. Contralateral eye should always be monitored (Reinstein et al., 2009b). Due to the fact that secondary glaucoma is associated with other ocular or systemic diseases, diagnosis should be directed toward the actual underlying problem (Lee and Higginbotham, 2005).

Tonometry is the diagnostic tool to measure the IOP. Normal canine IOP varies between 10 to 20 mmHg (Miller, 2008) regarding to type of instrument (tonometers). Not only IOP is circadian dependent (Martín-Suárez et al., 2014), its variation is noted among individuals as well as time of day, at which IOP is measured. There are three basic tonometers commonly used in veterinary practice; indentation, applanation and rebound tonometer.

Indentation tonometer (Schiotz tonometer; Gulden Ophthalmics, Pennsylvania, USA) measures the IOP as regards to the depth produced on the surface of the cornea by a load of known weight. The resulted IOP is inversely proportional to the reading number, while actual IOP must be obtained from a table of values. Placing this metallic device directly perpendicular to corneal surface makes this instrument less applicable in clinical practice. Applanation tonometer has been well recognized in veterinary field (Andrade et al., 2012). The most common instrument is TonoPen XL® (Reichert, Inc, New York, USA). It measures IOP by evaluating the amount of force required to flatten a constant area of the cornea, based on Imbert-Fick law (Park et al., 2011). Leiva et al. (2006) reported mean IOP measured by TonoPen XL® as 11.053 mmHg (SD 3.451 mmHg) whereas it was 11.6 mmHg (SD 2.7 mmHg), reported by Park et al. (2011)

Rebound tonometer is the most recent model of instrument that measures IOP by projecting a small probe at the corneal surface and analyzing characteristics of its rebound (Martinez-de-la-Casa et al., 2005). The speed of deceleration is measured and converted automatically by the device into pressure of the eye. This method does not require topical anesthetic prior to measurement. It is marketed under the name TonoVet® (Icare Finland, Helsinki, Finland) (Knollinger et al., 2005). Mean IOP measured by TonoVet® was 9.158 mmHg (SD 3.471 mmHg) (Leiva et al., 2006) whereas it was 16.9 mmHg (SD 3.7 mmHg) (Park et al., 2011)

To be able to differentiate between primary and secondary glaucoma, gonioscopy is a very useful technique to investigate iridocorneal angle (Miller, 2008). Even though glaucomatous eye may be unsuitable for gonioscopy due to the presence of corneal opacity, it is necessary for the fellow eye to be inspected when there is a potential risk for PACG to develop.

Treatment

In general, reduction in IOP can be achieved either by reducing the production of aqueous humor or increasing the facility of aqueous humor outflow. Glaucoma therapy can be approached by medication and surgery, although in many instances a combination of both is required.

Medical treatment *(table 1)*

Hyperosmotic agent: It reduces the production of aqueous humor by increasing osmotic concentration of blood perfusion to the eye. Dehydration of vitreous directly reduces vitreous volume and allows the intact lens to posteriorly shift, by which reducing pupillary block and facilitating outflow of aqueous humor (Scott and McLellan, 2013). The main indication for the use of hyperosmotic agents in canine glaucoma is to rapidly reduce marked high IOP as an emergency management of acute glaucoma (Reinstein et al., 2009a). Mannitol is a well-known osmotic diuretic that can significantly reduce IOP within 15 minutes of administration and remain effective for up to 6 hours (Lorimer et al., 1989).

Carbonic anhydrase inhibitors (CAI): Carbonic anhydrase is majorly responsible for synthesis of aqueous humor. It stimulates conversion of carbondioxide to bicarbonate, which in turn moving sodium and water into eye (Alward, 1998), forming aqueous humor. Carbonic anhydrase inhibitors reduce aqueous humor formation by inhibiting carbonic anhydrase enzymatic processes within the nonpigmented ciliary body epithelium (Skorobohach et al., 2003). Acetazolamide was the first systemic CAI commonly prescribed for people. Metabolic acidosis, described by anorexia, vomiting, diarrhea and panting, is the main adverse effect of systemic CAIs (Abrams, 2001). Recently, topical CAIs have extensively used for an attempt to reduce adverse effects of systemic CAIs. Dorzolamide (2%) and brinzolamide (1%) are commercially available at this present.

Prostaglandin analogues (PGs): Prostaglandin analogues act at prostanoid receptors by increasing relaxation of ciliary muscle while decreasing extracellular matrix surrounding ciliary muscle bundles. Thus, outflow of aqueous humor through uveoscleral pathway is enhanced. Due to specie differences of prostanoid receptors in

iris sphincter muscle, commercially available prostaglandin analogs cause an intense miosis in dogs while not in humans (Gelatt and MacKay, 2001). Miosis, in the meantime, helps to increase outflow through corneoscleral pathway in dogs.

Cholinergic agonists (Parasympathomimetic): Cholinergic agonist (pilocarpine hydrochloride) acts by stimulating parasympathetic receptors at neuromuscular junctions of iris sphincter muscle. It causes pupillary constriction and ciliary musculature contraction, both of which facilitate aqueous humor outflow through trabecular meshwork. (Bill and WåLINDER, 1966)

Beta adrenergic antagonist drugs (beta-blockers): Beta-blockers decrease aqueous humor production by reducing blood flow to ciliary body (Miller, 2008) via beta adrenergic receptors. It has been for years a drug of choice for glaucoma treatment in humans. The currently available drugs are timolol maleate, betaxolol hydrochloride, levobunolol hydrochloride, carteolol hydrochloride, and metipranolol. Beta blockers somehow enter blood circulation, causing cardiovascular effects (alterations in heart rate and rhythm, bronchoconstriction). Betaxolol hydrochloride is $beta_1$ selective blocker reported having fewer adverse effects (Frishman et al., 1994) although less effective in lowering IOP than nonselective drugs (Alward, 1998).

Sympathomimetics (Adrenergic agonists): Adrenergic agonists decrease production of aqueous humor by constricting ciliary body blood vessels, resulting in a decrease of ultrafiltration process (Alward, 1998). By stimulating alpha-adrenergic receptors, release of prostaglandin relaxes ciliary muscle bundles (Toris et al., 1999). Epinephrine is a nonspecific adrenergic agonist rarely used at this present because of significant systemic and ocular side effects. Therefore, brimonidine tartrate has been used as a selective and potent $alpha_2$-adrenergic agonist to lower IOP by reducing aqueous humor production and increasing uveoscleral outflow (Toris et al., 1995).

Table 1. Pharmacodynamics and clinical effects of hypotensive medications.

Medication	Pharmacodynamics & Generic drug	Clinical effect	
		DP	IO
Hyperosmotic agent	*Action*: Increase serum osmolality by reducing aqueous humor production and vitreous volume; reduce obstruction of outflow by posterior lens displacement *Drug*: Mannitol, Glycerol	√	√
Carbonic anhydrase inhibitor	*Action*: Inhibit carbonic anhydrase enzymatic processes, which in turn to decrease bicarbonate production and subsequently reduce aqueous humor synthesis *Drug*: Brinzolamide, Dorzolamide, Acetazolamide	√	
Prostaglandin analogues	*Action*: Increase relaxation of ciliary muscle and decrease extracellular matrix surrounding ciliary muscle; facilitate outflow of aqueous humor through uveoscleral pathway *Drug*: Latanoprost, Bimatoprost, Travoprost, Tafluprost		√
Cholinergic agonists (Parasympatho-mimetic)	*Action*: Stimulate parasympathetic receptors at neuromuscular junctions inducing pupillary constriction, ciliary musculature contraction, and facilitate outflow through trabecular meshwork *Drug*: Pilocarpine hydrochloride		√
Beta adrenergic antagonist	*Action*: Reduce blood flow in ciliary epithelial cells by blocking beta-receptors *Drug*: Timolol maleate, Betaxolol hydrochloride, Carteolol hydrochloride, Metipranolol, Levobulol hydrochloride	√	
Sympathomimetics (Adrenergic agonist)	*Action*: Reduce blood supply to ciliary body, thus decreasing ultrafiltration process, and also facilitate uveoscleral outflow via relaxation of ciliary muscle *Drug*: Epinephrine, Brimonidine tartrate, Oxymetazoline, Apraclonidine hydrochloride	√	√

Abbreviations: DP = Decrease aqueous humor production, IO = Increase aqueous humor outflow

Surgical treatment

Surgery would be considered when IOP has unsuccessfully controlled by medical therapy. Glaucoma surgery is based on two basic principles of treatment; increase aqueous humor outflow and decrease aqueous humor production.

Surgery to increase aqueous humor outflow: The techniques used in dogs aim to create an outflow for aqueous humor through drainage apparatus.

Filtering procedures: These procedures are such as cyclodialysis, iridectomy, iridencleisis, sclerotomy, sclerectomy, trabeculectomy, etc. Cyclodialysis is performed to separate ciliary body from sclera, hence creating communication between the suprachoroidal space and anterior chamber. It was reported to well maintain IOP at a desired level in dogs with secondary glaucoma to anterior uveitis (Lew and Lew, 2007). Iridectomy involved complete or peripheral removal of iris tissue (Playfair and Watson, 1979). It was recommended for primary acute angle-closure glaucoma. Iridencleisis is a procedure that radial section of iris is permanently positioned through limbal incision. From this, aqueous humor is drained into subconjunctival space beneath bulbar conjunctiva and slowly absorbed (Gelatt et al., 2011). Sclerotomy is used to gain access to the inner layer of the eye. Drainage channel that is created from the anterior chamber to the external surface of eye under conjunctiva allows aqueous humor to seep into a bleb from which it is slowly absorbed. There was a case report using a combination of iridencleisis and posterior sclerotomy as a surgical treatment of secondary glaucoma in dogs (Lew and Lew, 2009). Reduction of IOP was significant. IOP was maintained at a constant level during eight-month period following surgeries.

Trabeculectomy is a guarded filtering procedure creating an opening at sclera by removal part of trabecular meshwork. The opening is partially covered with a flap of tissue from sclera and conjunctiva. This new opening allows fluid to drain out of the eye, bypassing blocked drainage channels of trabecular meshwork. Typical complication following filtering procedures is healing of wound incision. Anti-fibrotic agents; such as Mitomicin-C, 5-Fluorouracil, were therefore considered by placing on site of surgery to reduce scaring during healing period (Kitazawa et al., 1991).

Anterior chamber shunt with gonioimplantation: This procedure allows aqueous humor drainage from anterior chamber to subconjunctival space through glaucoma drainage device (gonioimplant) (Hong et al., 2005). Basically, the drainage plate of the shunt is sutured to the sclera while the tube is inserted at the limbus into the anterior chamber. Various types of valve and non-valve gonioimplants were used in small animals (Abrams, 2001). Complications of shunting procedures included postoperative uveitis, occlusion of the gonioimplant with fibrin or blood in the anterior chamber, loss of gonioimplant and scarring of the filtering bleb (Renwick, 2002).

Surgery to reduce aqueous humor production (cyclodestruction): These procedures are performed to destroy portion of ciliary body, which is sufficient to bring the IOP down to be within normal limits (Fea et al., 2011).

Pharmacological ablation of ciliary body can be accomplished by injection of gentamicin and/or dexamethasone (Abrams, 2001) or cidofovir (Low et al., 2014) into vitreous cavity. This technique is recommended in blinded eyes. Common complications included inadequate control of IOP, hyphema, uveitis, retinal detachment, cataract development and phthisis bulbi (Reinstein et al., 2009a).

Cyclocryotherapy is another cyclodestructive method using cryogen to destroy ciliary processes. This procedure is undertaken to control the IOP by biphasic mechanism of intracellular ice crystal formation inducing ischemic necrosis of ciliary body (Shields, 1985). Liquid nitrogen and nitrous oxide are acceptable cryogens that were applied via the sclera (Sapienza, 2008). Because of high incidence of complications especially ocular pain (Reinstein et al., 2009a), cyclocryotherapy is not generally recommended in visual eyes (Fea et al., 2011).

Laser cyclophotocoagulation (CPC) has become more common as a procedure to destroy ciliary process via laser energy. This procedure can be performed using various types of laser at different wavelengths. Sources of laser that had been used for CPC included ruby (Beckman et al., 1972), argon (Kim and Moster, 1999), krypton (Raivio et al., 2001), Nd:YAG (Neodymium-doped yttirium aluminium garnet) (Lin et al., 2004) and diode (Egbert et al., 2001). In veterinary practice, laser CPC with the use of

ruby, argon, krypton are rare due to limited utilization, inconsistent outcome and severe complications (Mandal et al., 2009). Laser beam could be introduced to target cells via four major procedures; transpupillary, transvitreal, transscleral and endoscopic.

Transpupillary cyclophotocoagulation: Laser beam is delivered through wide pupil with the use of slit-lamp delivery system under indirect visualization via gonioscopic laser lens (Mandal et al., 2009). Either argon laser or Nd:YAG laser could be used for transpupillary CPC. Placement of contact lens on the sclera with slight indentation would improve visualization of ciliary processes. Transpupillary CPC however is rarely used nowadays because it requires clear visual axis and well-dilated pupil to enable photocoagulation of the entire length of ciliary processes (Pastor et al., 2001). Failure of transpupillary CPC was then related to the number of ciliary processes which could be visualized and treated (Shields, 1985).

Transvitreal endophotocoagulation: This method is performed in conjunction with vitrectomy. After anterior vitrectomy, endolaser probe is inserted directly to ciliary processes with visualization via operating microscope (Mandal et al., 2009; Fea et al., 2011). Argon and diode laser can be used (Pastor et al., 2001). Transvitreal endophotocoagulation would be considered in patients with clear media, aphakia or pseudoaphakia. Hemorrhage, transient choroidal detachment and hypotony were noted as post transvitreal endophotocoagulation complications (Patel et al., 1986).

Endoscopic cyclophotocoagulation (ECP): ECP is a technique that laser is directly introduced via a probe to directly photocoagulate ciliary processes under direct visualization (Spiess, 2012). Advantages of ECP include precised lower energy requirement, reduced inflammation to target tissues and reduced damage to collateral tissues (Lin et al., 2006). However, this procedure has not been commonly used because of invasiveness, requirement of operation theater, potential risk of damage to crystalline lens in phakic eye (Pastor et al., 2001) and risk for ocular infection (Mandal et al., 2009).

Transscleral cyclophotocoagulation (TSCP)

TSCP is a non-invasive laser technique, by which laser energy is transmitted through the sclera (figure 3) and then absorbed by melanin pigments in the outer layer of ciliary body epithelium (Pantcheva et al., 2007). This method of cyclodestruction occurs by photocoagulative thermal effect; reaction from linear absorption of laser energy by pigmented ciliary epithelial cells (Cantor et al., 1989). Following absorption, energy is converted into heat causing the temperature in target tissue rises rapidly. As tissue temperature has reached 42-62ºC (hyperthermia), blood vessels will be contracted and destroyed, resulting in tissue hypoxia and cell death. As tissue temperature increases to 60-100ºC, coagulation occurs leading to collagen contraction and protein denature. When tissue is superheated with temperature above 100ºC, vaporization of intra- and extracellular fluid that occurs would produce shockwave, observed as audible 'pop' sound (Berger and Eeg, 2006). When epithelium and stroma of pars plicata has undergone coagulation, aqueous humor can no longer be produced.

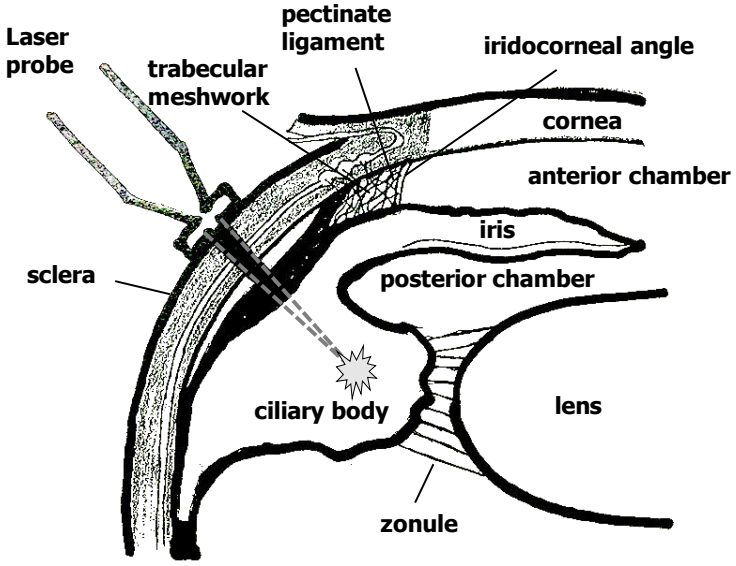

Figure 3. Transscleral diode lasser cyclophotocoagulation.

Two types of laser are widely used for TSCP; Nd:YAG and diode laser. Nd:YAG is a solid-state laser with wavelength of 1064 nm, while diode is a semiconductor laser emitting light energy of 810 nm (Youn et al., 1998). Both types of laser are well transmitted through sclera. Nevertheless, as a comparison of energy absorption by

melanin pigments, diode laser is preferred over Nd:YAG due to more targeted destruction with less inflammation (Schuman et al., 1990). Moreover, large and heavy Nd:YAG equipment unit required complex cooling system at all times. Therefore it was not really suitable for the use in veterinary practice (Spiess, 2012). Diode laser unit on the other hand, is portable and feasible without requirement of cooling system. The system comprises of source of diode energy, laser probe (optical fiber connected to a hand piece) and a footplate providing pre-measured distance.

Recently, the use of diode laser TSCP has been reported in animals (Cook et al., 1997; Nadelstein et al., 1997). There is lack of a uniform definition of success after TSCP though in terms of final IOP, success was defined as IOP was between 5 and 20 mmHg (Pastor et al., 2001). Several factors have brought into consideration for success of TSCP; laser energy setting, appropriate probe placement, individual (anatomical variation, type of glaucoma). Laser energy setting involves laser power (milliwatt: mW), exposure time (millisecond: ms), number of laser spots, energy per laser spot (joule = Watt × second), and total energy delivery (joule= Watt × second × number of laser spots). Balance of setting between power and exposure time is very important. Aggressive setting of 2250 mW and 2000 ms achieved 61% success in IOP control without retreatment (Noureddin et al., 2005). Topical hypotensive drug was significantly less required. However, intraocular hemorrhage, severe uveitis and sterile hypopyon were majorly observed. The setting protocol of relatively low power to short exposure time (1000 mW : 1000 ms) on the other hand reduced IOP by 50% (Mahmood et al., 2011). However, hypotensive medication was maintained as before TSCP.

Application of diode laser TSCP in primary canine glaucoma with laser setting of 1000 mW : 5000 ms showed 87.5% success in IOP control (Hardman and Stanley, 2001). All subjects no longer required topical hypotensive medications. Cataract was the major complication while hyphema gradually resolved within several days post TSCP. The study by (Hauber and Scherer, 2002) demonstrated that there was no correlation between success in IOP control and each laser setting factor. Increasing total amount of energy delivered per eye by increasing laser application sites

nonetheless played an important role in successful control of IOP. The greater number of application sites, the longer IOP control (Ueda et al., 2000).

As TSCP is performed without direct visualization of target cells, the exact location of pars plicata relative to external landmark must be known. In humans, TSCP was usually applied at a distance of 1.2- 1.5 mm posterior to the limbus (Pucci et al., 2003; Sha'aban and Asfour, 2004). In horses, laser probe was recommended at 4-6 mm posterior to the limbus (Miller et al., 2001) while it was suggested at 3-4 mm in canine eyes (Spiess, 2012). The distance from limbus to the posterior border of pars plicata in normal beagles was ranged from 4.08 ± 0.11 to 6.35 ± 0.1 mm (Ichihara et al., 2006). Thus, position of pars plicata was related to different size of globe among various species.

It was recommended by Spiess (2012) that a setting of 1500 mW : 1500 ms was suitable for small animals. Histological investigation of ciliary processes following various settings of diode laser TSCP protocols demonstrated that laser settings of 1500 mW : 1500 ms had pathological changes following coagulative necrosis manner (Morreale et al., 2007). A total energy of 2.25 Joules from this setting did not alter normal architecture of ciliary processes.

We herein have the TSCP protocol created as the treatment for canine absolute glaucoma. Laser setting was set a power of 1500 mW for the duration of 1500 ms at 64 spots, 360 degrees around the globe at a distance of 3, 4 and 5 mm posterior to the limbus. The setting was consistent to ensure strict uniformity of the treatment in all eyes.

Chapter 3

Materials and Methods

Animals

Forty three eyes from 36 glaucomatous dogs presented at the Ophthalmology Clinic, Small animal teaching hospital, Faculty of Veterinary Science, Chulalongkorn University were used in this study. All dogs were diagnosed as absolute glaucoma including 6 important criteria: (1) buphthalmic eyeball (figure 4), (2) uncontrolled IOP over 6 months duration (3) IOP higher than 35 mmHg, (4) unresponsive to medical hypotensive treatment, (5) no previous surgical treatment for glaucoma and (6) no specific ophthalmic complications; corneal ulcer, hyphema and intraocular infection.

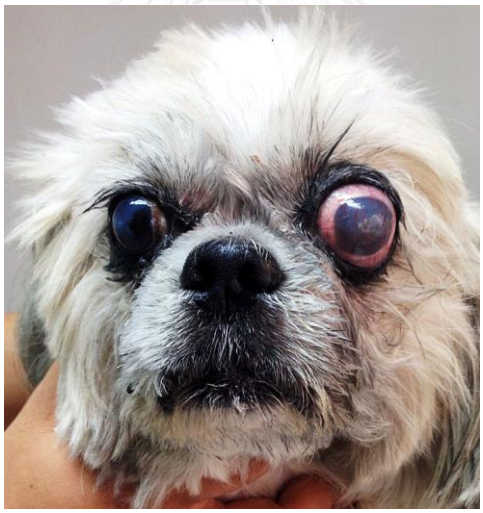

Figure 4. Left buphthalmic eye of an 8 year-old Shih Tzu with absolute glaucoma

Dogs had gone through ophthalmic examinations including menace response, dazzle reflex, pupillary light response, palpebral reflex, Schirmer tear test I, intraocular pressure measurement using a rebound tonometer (Tonovet®), fluorescein staining test and anterior chamber examination by slit lamp biomicroscopy (Kowa SL-15®; Kowa company. Ltd., Shizuoka, Japan). All procedures were performed under informed

consent provided by owners of each dogs. Trial procedures were approved by Chulalongkorn University Animal Care and Use Committee, Bangkok, Thailand. (No. 1431052)

Procedures

This study was divided into 3 parts.

Part 1. Anatomical study of pars plicata location

Part 2. Clinical study of TCSP

Part 3. Pathological study of pars plicata following TSCP

Part 1: Anatomical study of pars plicata location

Sample collection

Twelve buphthalmic eyes were derived from ten dogs, all of which their owners decided to treat glaucoma by removal of eyeballs. Complete blood counts and blood chemistry profiles (ALT, ALP, BUN, and creatinine) were assessed prior to anesthesia.

Anesthetic protocol Dogs were withheld water and food for 12 and 6 hours, respectively before anesthesia. Routine physical examinations were performed to evaluate body temperature, heart rate, respiratory rate, mucous membrane and hydration status. 25 mg/kg cefazolin sodium (Zefa M.H.®; M&H manufacturing Co. Ltd., Samutprakarn, Thailand) and 0.5 mg/kg dexamethasone sodium phosphate (Lodexa®; L.B.S.laboratory Ldt., Bangkok, Thailand) were systemically administered.

Dogs were premedicated with 0.2 mg/kg Diazepam (Diapine®; Atlantic labs, Bangkok, Thailand) and 4 µg/kg Fentanyl citrate (Fentanyl Kern Pharma®; Kern Pharma S.L., Terrasa, Spain) intravenously. General anesthesia was induced by 4 mg/kg of 1% propofol (Lipuro®; B. Braun, Melsungen, Germany) intravenously. During operation, dogs were maintained with 2% isoflurane (Aerrane Isoflurane USP®; Baxter Healthcare of Puerto Rico, Puerto Rico, U.S.A.) delivered in oxygen. All dogs received lactated ringer's solution intravenously at the rate of 10 ml/kg/h until fully recovered.

Surgical procedure Following standard aseptic technique, periocular skin, conjunctival fornix and ocular surface were prepared. Transpalpebral enucleation was performed (figure 5). Elliptical full-thickness skin incision approximately 5 mm distance from eyelid margins was created. Blunt dissection of conjunctiva and Tenon's capsule followed by transection of extraocular muscles were performed around the globe. Once approaching toward the posterior wall of eyeball, the optic nerve was transected and then, the eyeball was removed. Extraocular muscles were sutured to reduce dead space in the eye socket. 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. 25 mg/kg cephalexin monohydrate (Sialexin®; Siam Bheasach, Bangkok, Thailand) had been orally administered for 10 days after surgery. Skin sutures were removed on 10 days postoperatively. Post-operative pain was control with 4 mg/kg tramadol hydrochloride (Tramal®; Aayush Food&Herbs limited, Karnataka, India) orally 3-5 days.

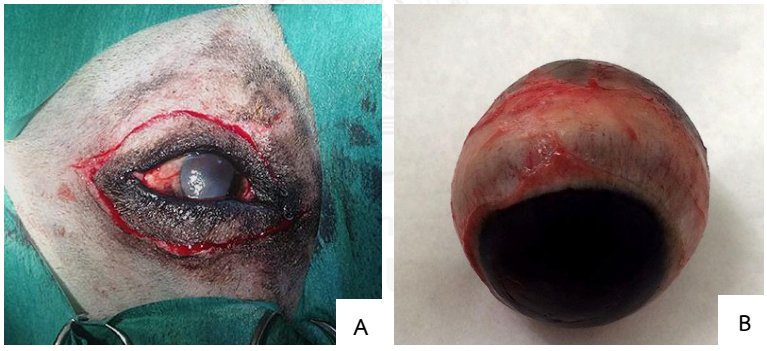

Figure 5. Removal of an eyeball by transpalpebral enucleation.
A) Elliptical full-thickness incision around the eye; B) Buphthalmic eyeball removed.

Data collection

All enucleated eyeballs were identified as right or left, and then marked at 12.00 position by suture material (figure 6A). After extraocular muscles around the globe were removed, eyeballs were immediately immersed in 4% paraformaldehyde in PBS for 1 hour. Each eyeball was sectioned in a coronal plain at 8 mm distance from limbus by a straight razor blade (figure 6B); posterior segment of the eye was discarded. Disposing of vitreous body and lens allowed direct visualization of ciliary body in the anterior segment of the eye (figure 6C). To measure distances (millimeter) of pars plicata from limbus at 12 positions clockwise, 26 gauge needles were internally pierced through sclera at the anterior and posterior boundary of pars plicata (figure 6D). Electronic digital caliper (Powerfix®, Milomex Ltd., Bedfordshire, UK) was used to measure distance from limbus to the anterior boundary of pars plicata (figure 6E) and distance from limbus to the end of pars placation (figure 6F).

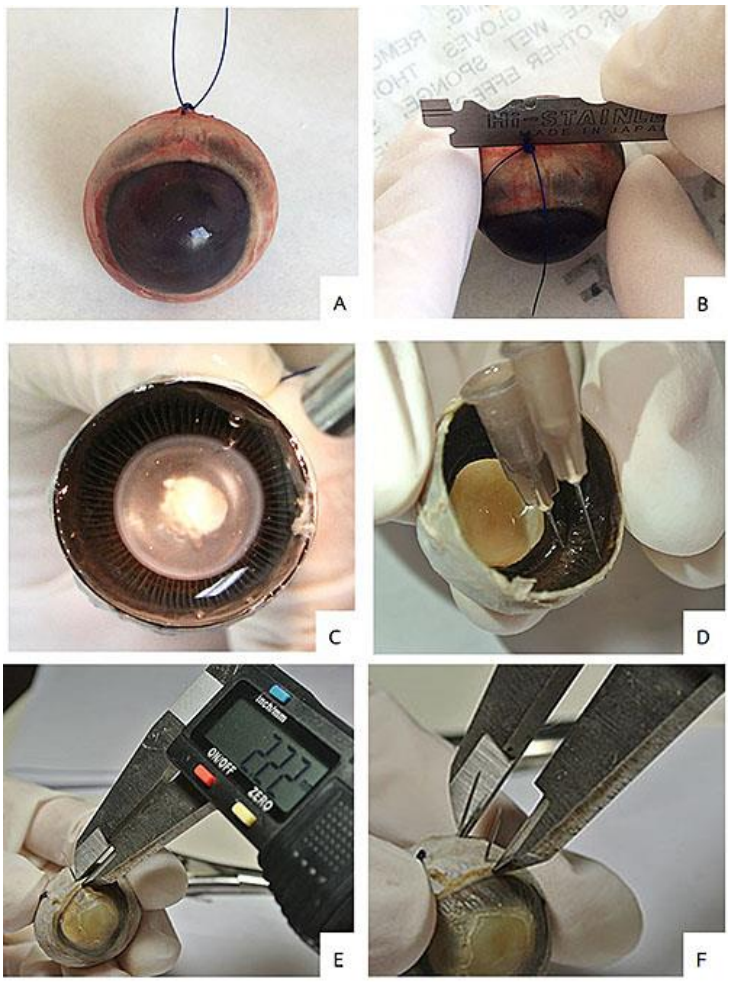

Figure 6. Determination of pars plicata location.

A) 12.00 position of eyeball located; B) Globe vertically cut into bisection; C) Anterior segment of the eye demonstrating pars plication; D) Two 26 gauge needles indicating anterior and posterior boundary of the pars plicata; E) Distance from limbus to the anterior boundary of pars plicata measured; F) Distance from limbus to the posterior boundary of pars plicata measured.

Part 2: Clinical study of TCSP

Sample collection

Twenty six dogs (31 eyes) were included in part 2 of the study. Three days prior to TSCP, dogs were prescribed 0.3 mg/kg prednisolone orally and 0.3% topical

tobramycin eye drop (Tobrex® ophthalmic solution; Alcon-Couvreur, Puurs, Belgium) four times daily. On TSCP day, routine ophthalmic examinations, including tonometry with rebound tonometer (figure 7) were performed. B-scan ocular ultrasonography was performed using ocular ultrasonographic machine (Ultrascan® Imaging system; Alcon Laboratories, Inc, Hünenberg, Switzerland) (figure 8). From B-scan ultrasonographic image, axial globe length (millimeter) was electronically measured along visual axis from cornea to the posterior wall of the eye.

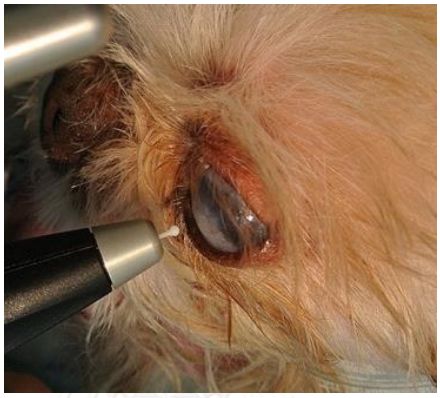

Figure 7. Rebound tonometry in absolute glaucomatous eye

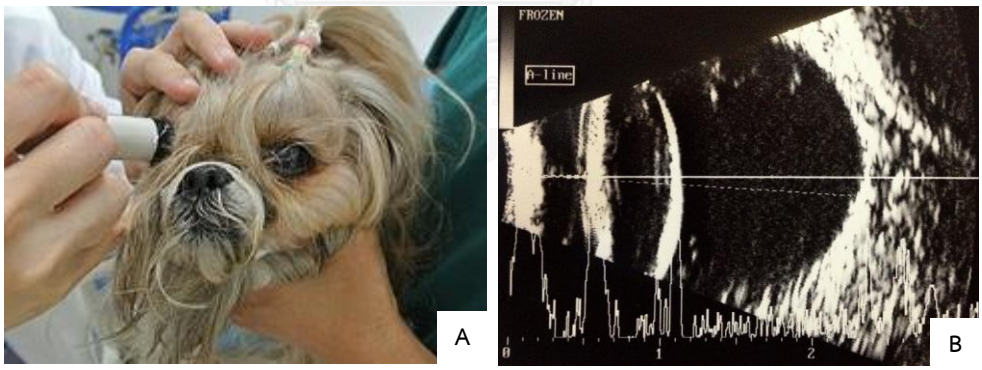

Figure 8. Ophthalmic examinations in canine glaucoma.
A) B-scan ocular ultrasonography; B) Measurement of axial globe length

Anesthetic protocol Dogs were withheld food and water for 12 and 6 hours, respectively before anesthesia. Routine physical examinations were performed to evaluate body temperature, heart rate, respiratory rate, mucous membrane and hydration status. 25 mg/kg ceftriaxone sodium (Trixophin®; Shenzhen zhijun

pharmaceutical. Co., Ltd., Shenzhen, China), 0.5mg/kg dexamethasone sodium phosphate and 4 mg/kg tramadol hydrochloride (Tramache®; Harson Laboratories, Baroda, India) were systemically administered.

Dogs were premedicated with 0.2 mg/kg Diazepam and 4 µg/kg Fentanyl citrate intravenously. General anesthesia was induced by 4 mg/kg of 1% propofol intravenously. During TSCP, dogs were maintained with 2% isoflurane delivered in oxygen. All dogs received lactated ringer's solution intravenously at the rate of 10 ml/kg/h until fully recovered.

Transscleral diode laser cyclophotocoagulation procedure

Diode laser TSCP was performed in dogs under general anesthesia using an 810-nm diode laser (DioVet Laser System®; IRIDEX, Mountain View, USA) with laser energy being delivered via glaucoma probe (G-probe) (figure 9). A setting of 1500 mW power and 1500 ms laser exposure time resulted in a total energy delivery of 2.25 Joule/site. G-probe was positioned perpendicular to the globe with slight indentation on the conjunctiva. Laser was applied at 3, 4 and 5 mm posterior to the corneal limbus (figure 10A), at 64 sites; 360 degrees around the eyeball (figure 10B and 11A). Applications at 3.00 and 9.00 position clockwise were avoided. IOP was immediately measured as soon as TSCP was finished. To reduce IOP to below 15 mmHg, anterior chamber paracentesis was performed using a 26 gauge needle inserted into the anterior chamber at the corneal limbus to release small amount of aqueous humor (figure 11B). After that, 0.8 mg dexamethasone sodium phosphate and 2 mg gentamicin sulfate was subconjunctivally injected (figure 11C) with divided amount to the upper and lower conjunctiva. Tobramycin ointment (Tobrex® ophthalmic ointment; Alcon-Couvreur, Puurs, Belgium) was topically applied, followed by temporary partial tarsorrhaphy (figure 11D). Palpebral fissure at the nasal region was slightly being apart to allow measurement of IOP post laser.

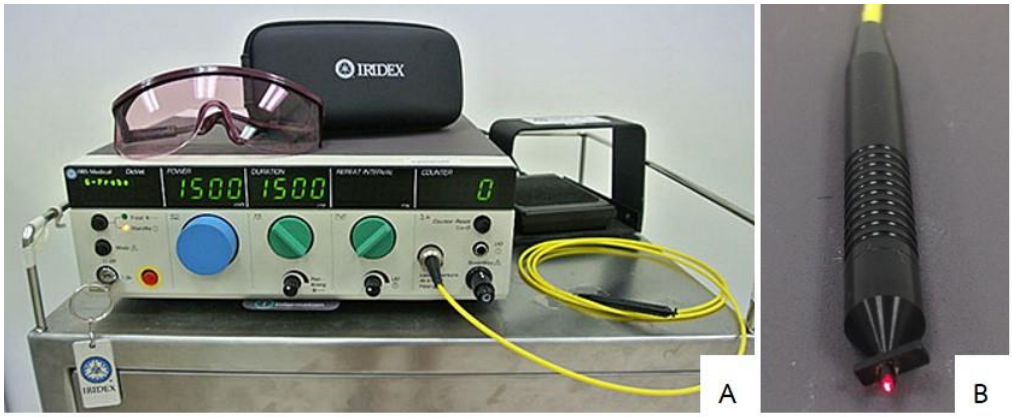

Figure 9. A photograph of the 810-nm diode laser unit.

A) A setting of 1500 mW power and 1500 ms duration of laser time; B) G-probe for transscleral diode laser cyclophotocoagulation in veterinary practice.

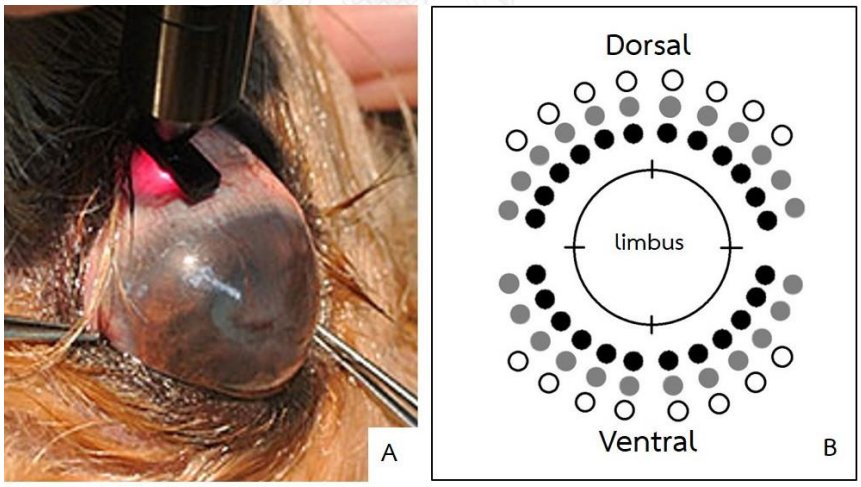

Figure 10. Locations of TSCP 360 degrees around the eye.

A) Tip of the G-probe perpendicularly placed with slight indentation on the upper conjunctiva (note that aiming beam is in active mode); B) locations of laser applications for a total of 64 spots around the eyeball (note that solid circle, soft circle and white circle representing application of probe at 3, 4 and 5 mm posterior to the limbus, respectively).

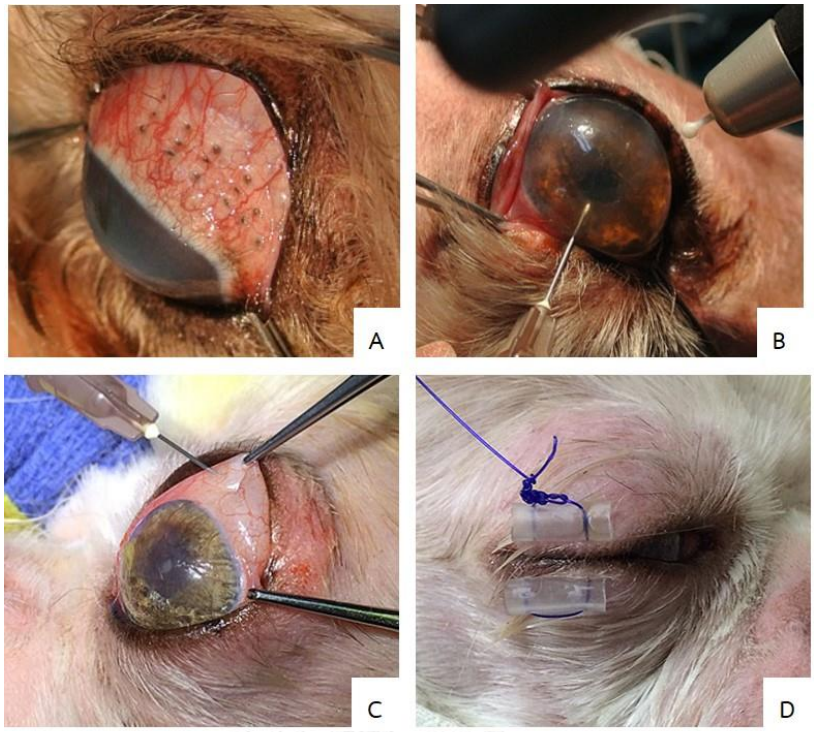

Figure 11. TSCP procedures.

A) Locations on the conjunctiva where diode laser was introduced via a G-probe; B) Anterior chamber paracentesis; C) Subconjunctival injection; D) temporary partial tarsorrhaphy (note that hollow plastic cylinders were placed to prevent stitch bites).

Post TSCP, systemic administration included 25 mg/kg ceftriaxone sodium, 0.5 mg/kg dexamethasone sodium phosphate and 4 mg/kg tramadol hydrochloride were subcutaneously administered for the first 4 days. After that ceftriazone sodium was replaced by 25 mg/kg oral cephalexin monohyrate for 5 days, whereas dexamethasone sodium phosphate was replaced by 0.5 mg/kg oral prednisolone that was continued for 2 weeks with a withdrawing dose. Meanwhile 0.3% tobramycin was topically given four times per day until tarsorrhaphy was removed at 3 weeks post TSCP. As soon as fluorescein staining test was found negative on the cornea, a combination of 0.3% tobramycin with 0.1% dexamethazone eyedrop (Tobradex® ophthalmic solution; Alcon-Couvreur, Puurs, Belgium) was topically given twice daily. To lubricate ocular surface, artificial tear (Systane ultra®; Alcon laboratories, Inc. Fort Worth, USA) was

considered when STT 1 value was below 12 mm wetness. Topical hypotensive drugs were continued with adjusted dose by time.

Data collection

Any 'pops' sounds which occurred during treatment, were noted. IOP was measured before TSCP and then following TSCP at day 1, 2, 3, 4, 7, 14, 21, 28, 42, 56, 77 and 105. Ocular complications were recorded via routine ophthalmic examinations. Axial globe length was measured before TSCP and day 105 post TSCP. Number of topical hypotensive drug application was recorded at the end of the study.

Part 3. Pathological study of pars plicata following TSCP

Sample collection

Six buphthalmic eyes were derived from six dogs, all of which their owners decided to treat glaucoma by removal of eyeballs. Eyes were randomly assigned into 3 groups; group 3.1 received TSCP at dorsal part of the eyeball, group 3.2 received TSCP at ventral part of the eyeball, and group 3.3 did not receive TSCP. Following diode laser TSCP in group 3.1 and 3.2, transpalpebral enucleation was performed (as described in materials and methods part 1).

Procedure Complete blood counts and blood chemistry profiles were assessed prior to anesthesia. Anesthesia and animal preparations were conducted (as described in materials and methods part 1). Diode laser TSCP was performed using the same equipment and protocol setting as described in materials and methods part 2. Laser applications were applied at 32 sites/eyes in each group of samples (figure 12). Dogs in group 3.1 received laser at 3, 4 and 5 mm posterior to limbus approximately 180 degrees on the dorsal part of the eye, while those in group 3.2 received the same numbers of laser application, at the same distances from limbus but to the ventral part of the eye. After TSCP, followed by enucleation, 25 mg/kg cefazolin sodium and 0.5mg/kg dexamethasone sodium phosphate were systemically administered to all dogs and continued with 25 mg/kg cephalexin monohydrate orally until skin sutures were removed on day 10 postoperatively. Post-operative pain was control with 4 mg/kg tramadol orally 3-5 day.

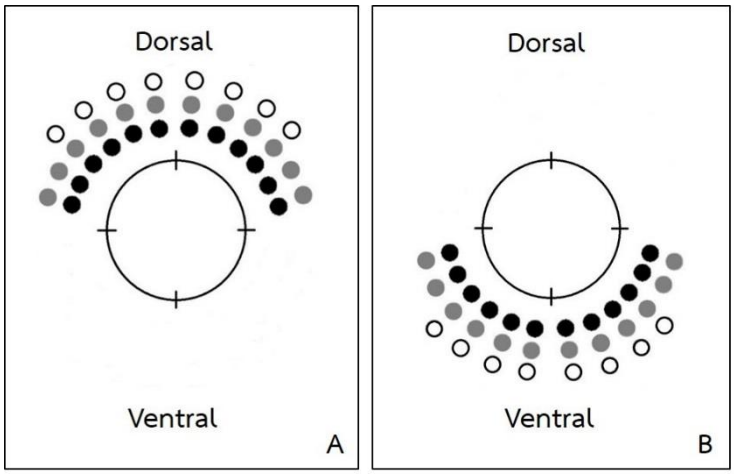

Figure 12. Locations of TSCP for pathological study.
A) Sites of laser application in group 3.1; B) Sites of laser application in group 3.2. (Note that solid circle, soft circle and white circle representing application of G-probe at 3, 4 and 5 mm posterior to the limbus, respectively).

Data collection

Each half of enucleated eyeball was indicated, then immediately immersed in 4% paraformaldehyde in PBS for 1 hour. All eyeballs were sectioned via a coronal plain at 8 mm distance from limbus by a straight razor blade. After vitreous body and lens being disposed, pathological changes of pars plicata and surrounding area were macroscopically investigated.

Anterior segments of the eyes were continuously fixed in the same type of fixative for 24 hours. Routine histological process was performed and the fixed segment was embedded in paraffin wax. After that, sample was vertically sectioned over the ciliary position at 4 μm thickness and stained with hematoxilin and eosin (H&E) staining. The microscopic lesions were evaluated under light microscope.

Data and statistical analysis

Part 1. Anatomical study of pars plicata location

Mean of distances from limbus to the anterior and the posterior boundary of pars plicata were calculated with standard deviation at each 12 locations clockwise. Width of pars plicata was calculated from a subtraction between distance from limbus

to the posterior boundary of pars plicata and that of distance from limbus to the anterior boundary. (figure 13)

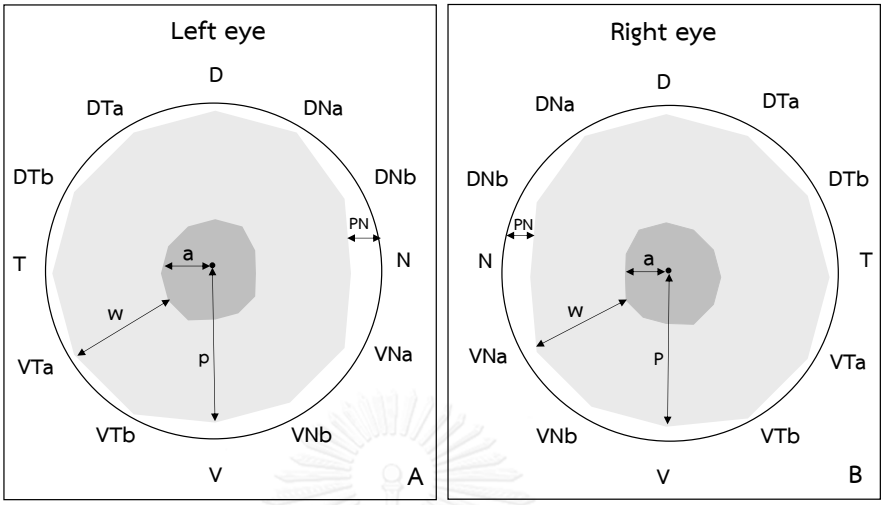

Figure 13. Schematic diagrams illustrating distances measured in part 1 of the study. A) Left eye; B) Right eye.

a = distance from limbus to the anterior boundary of pars plicata

p = distance from limbus to the posterior boundary of pars plicata

w = width of pars plicata, pn = portion of pars plana

D = dorsal (12:00 on both eyes) , V = ventral (6:00 on both eyes)

DNa = dorsonasal (1:00 on the left eye/ 11:00 on the right eye)

DNb = dorsonasal (2:00 on the left eye/ 10:00 on the right eye)

N = nasal (3:00 on the left eye/ 9:00 on the right eye)

VNa = ventronasal (4:00 on the left eye/ 8:00 on the right eye)

VNb = ventronasal (5:00 on the left eye/ 7:00 on the right eye)

VTb = ventrotemporal (7:00 on the left eye/ 5:00 on the right eye)

VTa = ventrotemporal (8:00 on the left eye/ 4:00 on the right eye)

T = temporal (9:00 on the left eye/ 3:00 on the right eye)

DTb = dorsotemposal (10:00 on the left eye/ 2:00 on the right eye)

DTa = dorsotemposal (11:00 on the left eye/ 1:00 on the right eye)

Part 2. Clinical study of TCSP

Six parameters were investigated and analyzed.

- Numbers of audible 'pop' sound: Numbers of 'pop' sound recorded at laser sites were calculated into percentage in four quadrants of the eye.
- IOP: Mean IOP ± SE was calculated at all-time points to statistically compare between before and after TSCP. Repeated measured ANOVA was applied with a significant level at $p<0.05$ using SPSS program (version 17, IBM Corporation, Armonk, New York, USA).
- Qualified success of TSCP to control IOP: It was defined as a final IOP was less than or equal to 20 mmHg at the end of the study.
- Numbers of topical hypotensive drug administration: Numbers of topical application per day were calculated into mean ± SE at each time point of clinical follow-up.
- Axial globe length: Mean axial globe length ± SE was calculated and statistically compared between before and after TSCP. Repeated measured ANOVA was applied with a significant level at $p < 0.05$ using SPSS program (version 17, IBM Corporation, Armonk, New York, USA).
- Clinical complications: Complications that occurred post TSCP were recorded and descriptively analyzed at each time point of clinical follow-up.

Part 3. Pathological study of pars plicata following TSCP

Pathological changes at pars plicata and surrounding area were macroscopically and microscopically compared among 3 groups of samples in descriptive manner.

Chapter 4

Results

Part 1: Anatomical study of pars plicata location

Twelve eyeballs were derived from ten dogs; Shih Tzu (6), Poodle (2), Miniature Pinscher (1) and mixed breed dog (1).

From the right eye (figure 14), distance from limbus to the anterior border of pars plicata ranged from 1.09 ± 0.5 to 1.83 ± 0.48 mm around the clock while the distance from limbus to the posterior border ranged from 4.17 ± 0.42 to 5.78 ± 0.91 mm. The nearest location of anterior boundary of pars plicata was shown at 9.00 clock position, whereas the farthest was at 1.00 clock position. The nearest and farthest distances to the posterior boundary of pars plicata were also observed at the same clock position as they were for the anterior boundary. Subtraction of distance from limbus to anterior boundary out of distance from limbus to posterior boundary revealed width of pars plicata. The widest portion of pars plicata of the right eye was at 3.00 clock position whereas the narrowest portion remained at 9.00 clock position. From the left eye (figure 15), distance measured from limbus to the anterior border of the pars plicata was ranged from 1.53 ± 0.32 to 2.33 ± 0.5 mm whereas that of measured from limbus to the posterior border of the pars plicata was ranged from 4.71 ± 0.51 to 6.74 ± 0.7 mm. The nearest location of anterior boundary of pars plicata from the limbus was at 4.00 clock position, while the farthest location was at 12.00 clock position. In the meantime, the nearest location of posterior boundary of pars plicata from the limbus was also at 3.00 clock position. The farthest location of posterior boundary of pars plicata from the limbus was at 11.00 clock position, which was in the dorsotemporal region.

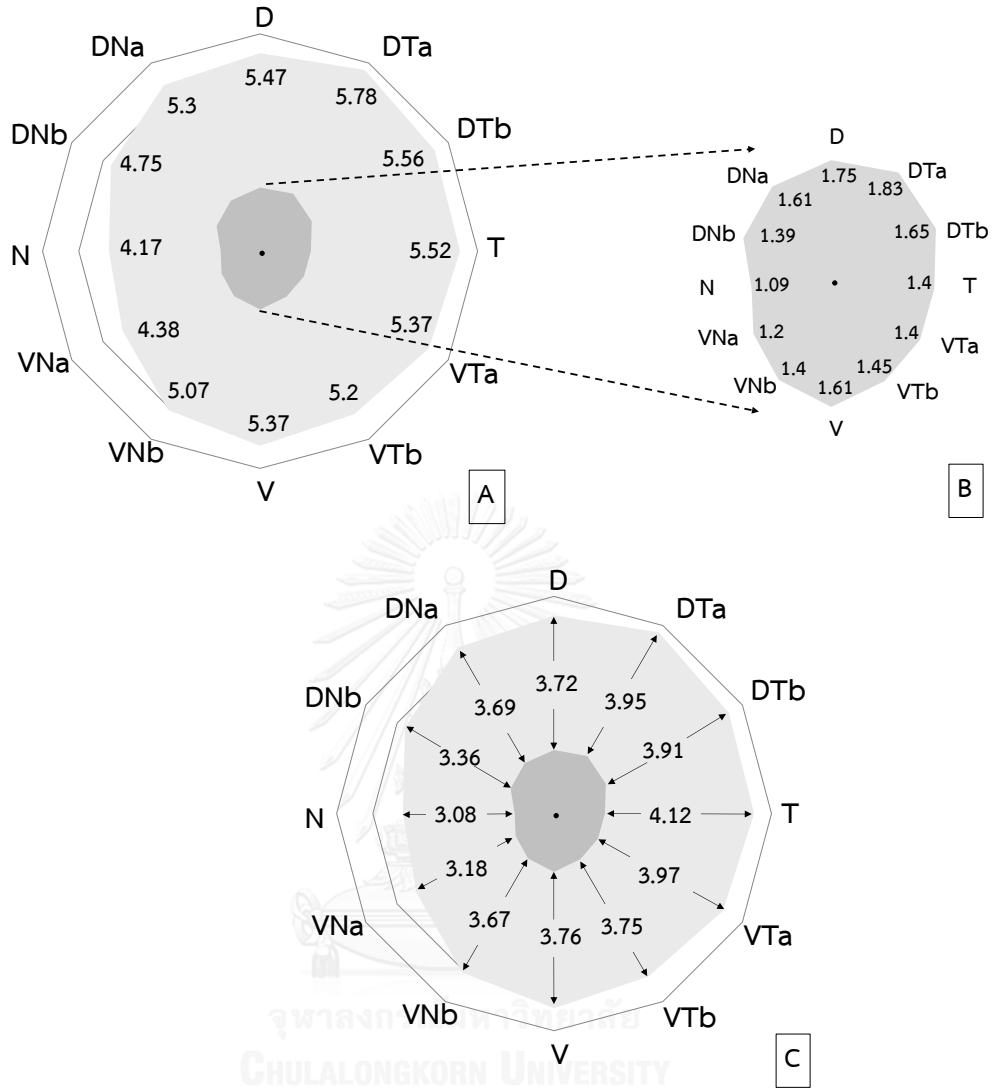

Figure 14. A schematic diagram of the anterior segment of the right canine eyecup demonstrating mean distances (millimeter) measured.

A) posterior border of pars plicata from limbus; B) anterior border of pars plicata from limbus; C) width of pars plicata. Note that dark spot at center = limbus

D = dorsal, T = temporal, V = ventral, N = nasal, DTa = dorsotemporal (1.00), DTb = dorsotemporal (2.00), VTa = ventrotemporal (4.00), VTb = ventrotemporal (5.00), DNa = dorsonasal (11.00), DNb = dorsonasal (10.00), VNa = ventronasal (8.00) and VNb = ventronasal (7.00)

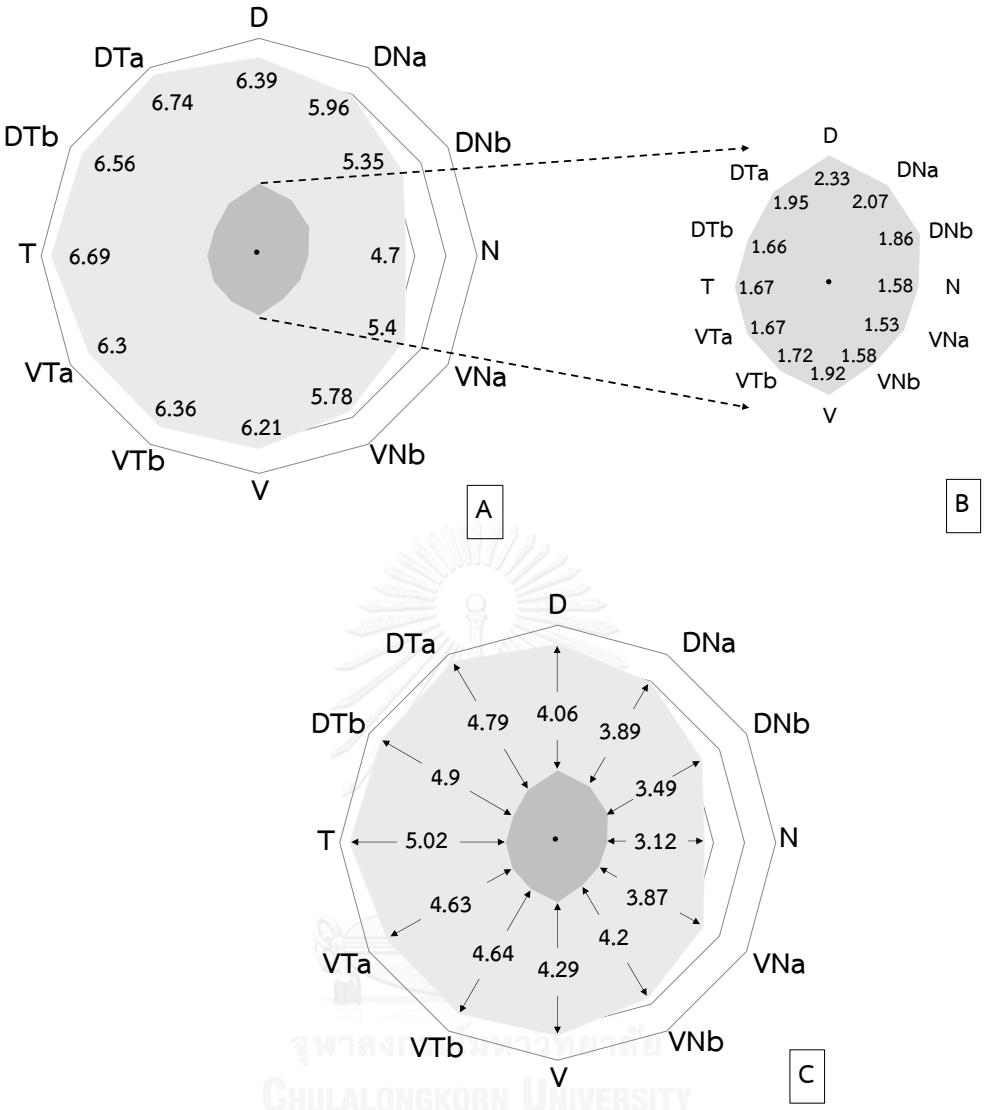

Figure 15. A schematic diagram of the anterior segment of the left canine eyecup demonstrating mean distances (millimeter) measured.

A) posterior border of pars plicata from limbus; B) anterior border of pars plicata from limbus; C) width of pars plicata. Note that dark spot at the center = limbus

D = dorsal, T = temporal, V = ventral, N = nasal, DTa = dorsotemporal (11.00), DTb = dorsotemporal (10.00), VTa = ventrotemporal (8.00), VTb = ventrotemporal (7.00), DNa = dorsonasal (1.00), DNb = dorsonasal (2.00), VNa = ventronasal (4.00) and VNb = ventronasal (5.00)

Locations of laser application were confirmed in canine buphthalmic eyeball by three needles being pierced through the globe at 3, 4 and 5 mm posterior from the limbus (figure 16). As indicated by three needles, it was demonstrated from the anterior half of the eyeball that width of pars plicata was covered by laser treatment.

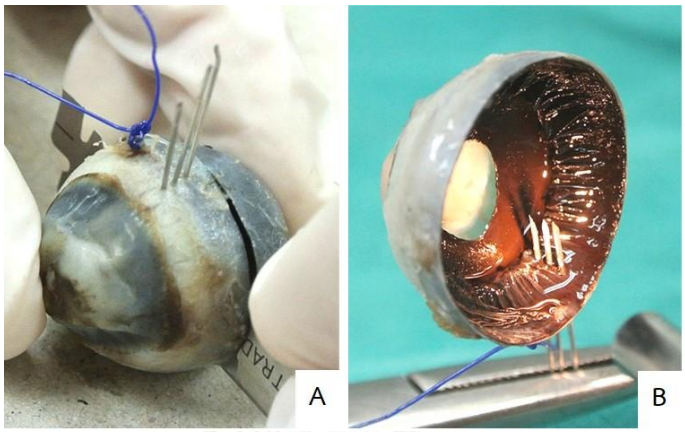

Figure 16. Locations of laser application.
A) three needles representing locations of laser probe at 3, 4 and 5 mm posterior to the limbus; B) Locations of needles pierced through the sclera at 3, 4, 5 mm posterior to the limbus.

Part 2: Clinical study of TCSP

Twenty six absolute glaucomatous dogs (31 eyes) that were included into part 2 of the study were Shih Tzu (n=13), Poodle (n=2), Siberian husky (n=1), Chihuahua (n=1), Yorkshire (n=1), Pomeranian (n=1) and mixed breed (n=7). Sixteen dogs were female while ten dogs were male with an average age of 9.45 years (1-13 years). TSCP were treated on the right eye in 9 dogs; left eyes in 12 dogs and both eyes in 5 dogs. Four eyes had narrowing of palprebral fissure. Lateral cantotomy was therefore required prior to TSCP to allow ease of probe application. Scleral perforation occurred in two eyes immediately after laser application (figure 23A). Aqueous humor leaked into subconjunctival space causing difficulty in perpendicularly applying a laser probe.

Of 360 degrees around the globe, audible popping sounds were 71.97%. Percentage of pop sound from all eyes was ranged from 50% to 96%. The highest number of pop sound was recorded in ventrotemporal quardrant (figure 17). At the dorsal part of the eye, its percentage was comparable between temporal and nasal quadrant. Ventronasal quadrant showed the least number of pop sound among others. Number of pop sound had no statistically significant difference between each quadrant.

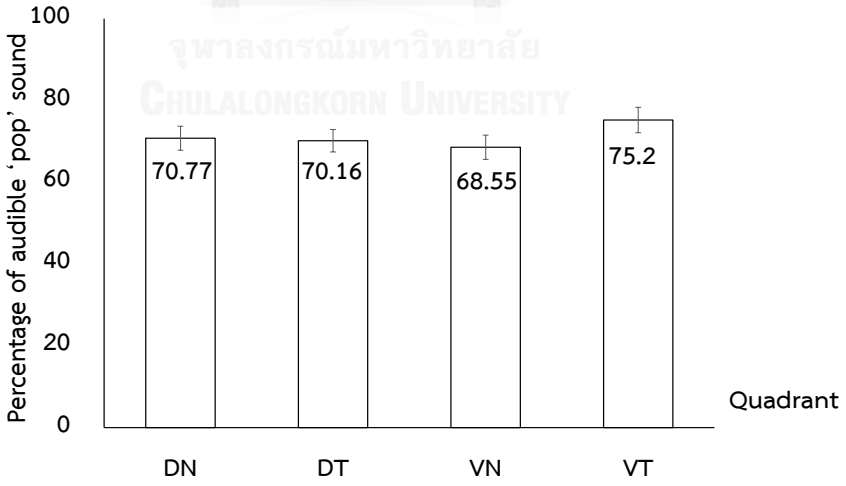

Figure 17. Percentage of audible 'pop' sound calculated in each quadrant of the eye (n=31).

Note that DN=dorsonasal; DT= dorsotemporal; VN=ventronasal; VT=ventrotemporal.

Maximal number of pop sound was recorded at a distance of 3 mm from limbus (figure 18). The minimal number of pop sound was at 5 mm distance, which was statistically low compared to the others.

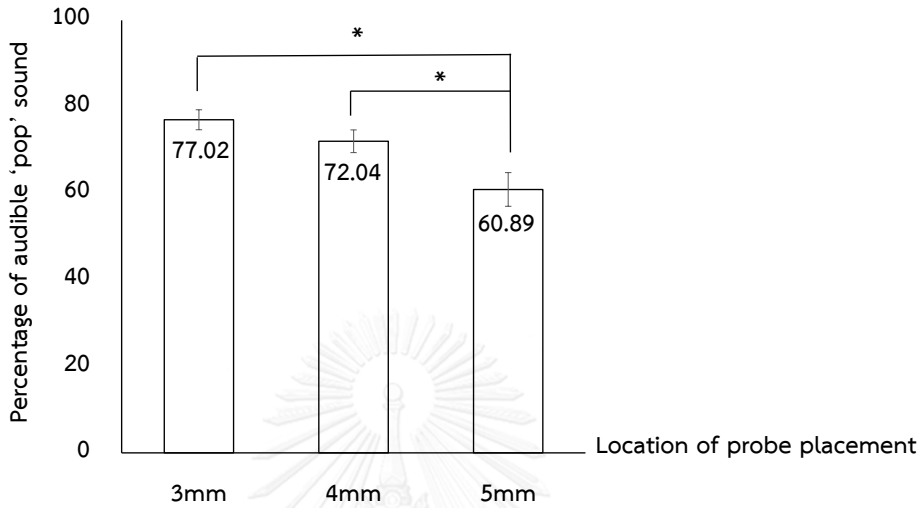

Figure 18. Percentage of audible 'pop' sound calculated at each distance of laser probe application (n=31).

Note that 3mm, 4mm and 5mm representing distances in millimeter from corneal limbus. (Asterisk sign indicates significantly difference of percentage of audible 'pop' sound at *p<0.05*)

Mean IOP at pre TSCP was 62.1 ± 2.45 mmHg (range = 35 to 98 mmHg). An immediate postoperative IOP rise was noted following TSCP in most cases (29/31 eyes; 93.5%), an average of elevation was 40.86 ± 4.12 mmHg. Day 1 post TSCP, mean IOP statistically reduced to 15.69 ± 2.05 mmHg. IOP spike during 24 hours after TSCP was noted in only one dog. After that mean IOP gradually decreased to 9.4 ± 1.39 mmHg at day 7 post TSCP, then being maintained at a level below 10 mmHg throughout the study (figure 19). Post TSCP, mean IOP was statistically low at all-time points as compared to that of pre TSCP. Mean IOP at the end of the study was 6.58 ± 0.95 mmHg.

During day 42 to day 105, IOP exceeding 20 mmHg was observed in six dogs, five of which had gradual reduction of IOP thereafter. Except for one eye that IOP was being maintained at 23.5 mmHg at the end of the study. Qualify success in controlling IOP in this study was 96.8%.

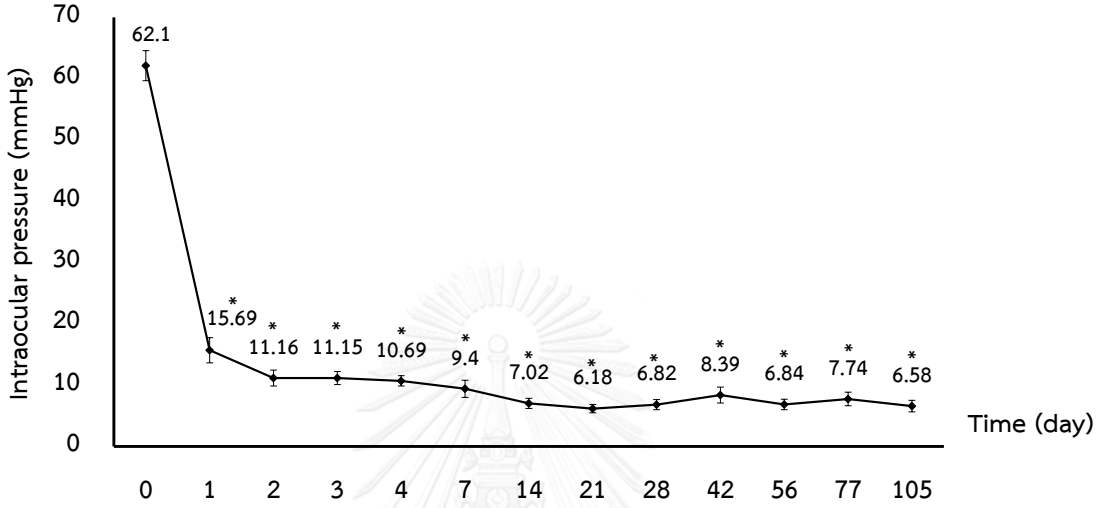

Figure 19. Mean intraocular pressure (mmHg) ± SEM at pre/post TSCP.
(Asterisk sign indicates significantly difference of IOP reduction at *p<0.05*)

Multiple groups of topical hypotensive drugs were applied in all dogs. They were β-blockers, carbonic anhydrase inhibitors, prostaglandin analogs and α_2-adrenergic agonists. Some dogs received single medication whereas others received fixed combination of drug. Pre TSCP, mean number of topical hypotensive application was 8.9 ± 2.2 drops per day (range = 8-15 drops). Number of topical hypotensive drugs were continued and adjusted in accordance with IOP (figure 20). Reduction of eye drops began at day 14 post TSCP. Significant reduction of number of topical hypotensive application was first observed at day 21. After that, mean number of drops was gradually declined with significant difference as compared to pre TSCP.

At the end of study, mean number of topical applications was 2.97 ± 2.4 drops per day. Five eyes no longer required topical medications. Two eyes had to continue the same number of hypotensive medications as compared to pre TSCP in order to control IOP.

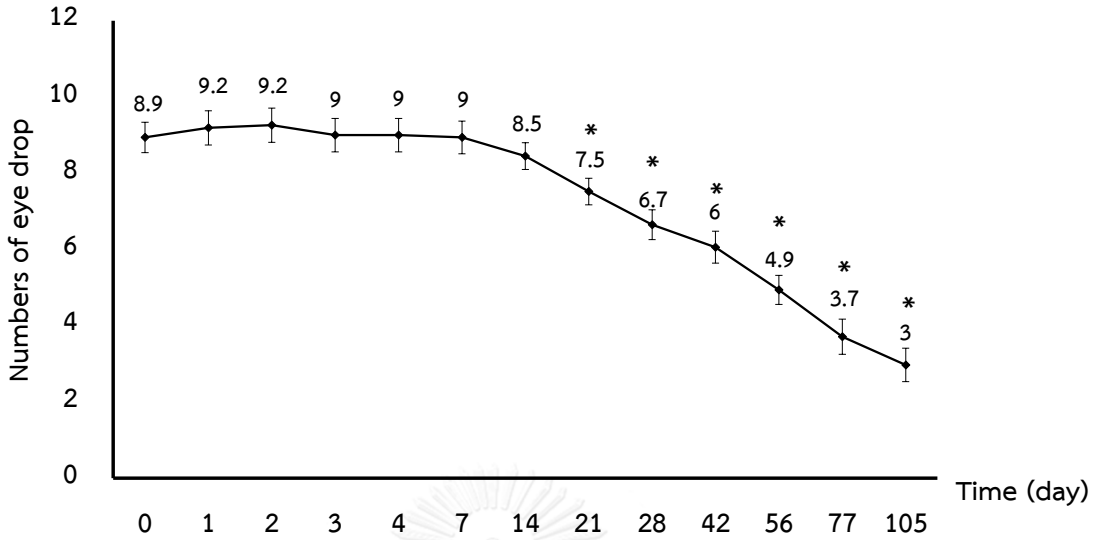

Figure 20. Mean number of hypotensive drug applications (per day) ± SEM at pre/post TSCP.

(Asterisk sign indicates significantly difference of number of drug application at *p<0.05*.)

Pre TSCP, mean axial globe length of absolute glaucomatous eyes was 21.62 ± 0.32 mm, while that of the other eyes, which were non-glaucomatous, was 18.68 ± 0.26 mm. At the end of the study, the length in TSCP-treated eyes significantly decreased to 18.63 mm. ± 0.39 mm, which was not statistically different as compared to that of in non TSCP-treated eyes (figure 21).

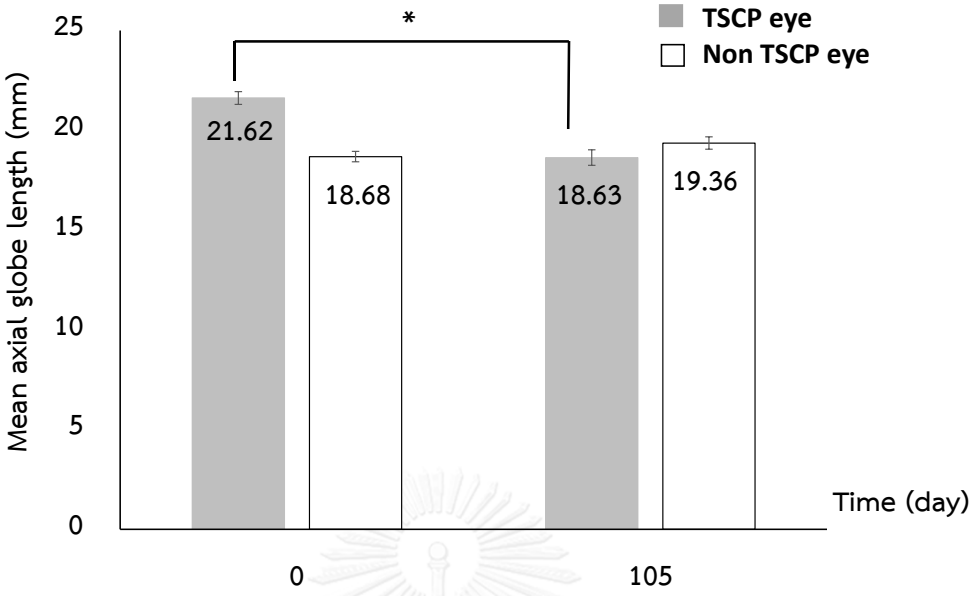

Figure 21. Mean axial globe length (mm) ± SEM at pre/post TSCP.

(Asterisk sign indicates significant difference of mean axial globe length at $p<0.05$.)

Post TSCP, ocular discomfort was observed in few cases. The rest of palpebral fissure could widely open. A loose of suture material of lateral tarsorrhaphy began during day 8 to day 18. In one eye that hyphema occurred immediately after paracentesis, it resolved in 3 days afterward. Black burns at sites of TSCP were observed in some cases, some of which were permanently visible as pinpoint black spots at the end of the study (figure 23B).

Post-operative complications were observed in 18/31 eyes (figure 22). They appeared at different post laser timing. For the period of one week post TSCP, mild aqueous flare was noticed in few eyes. Fibrin that was observed in the anterior chamber (3/31) resolved within day 5. Superficial corneal ulcer (12/31) appeared at the center of the eye, most of which was observed at day 3-5. Six of twelve ulcerative eyes were considered complicated corneal ulcer. When noticed, tarsorrhaphy was removed and debridement and/or krid keratotomy was performed. In the other six eyes with simply ulcer (approximately 1 mm in diameter), topical antibiotic was applied more often. After tarsorrhaphy was removed, simple ulcers were completely healed.

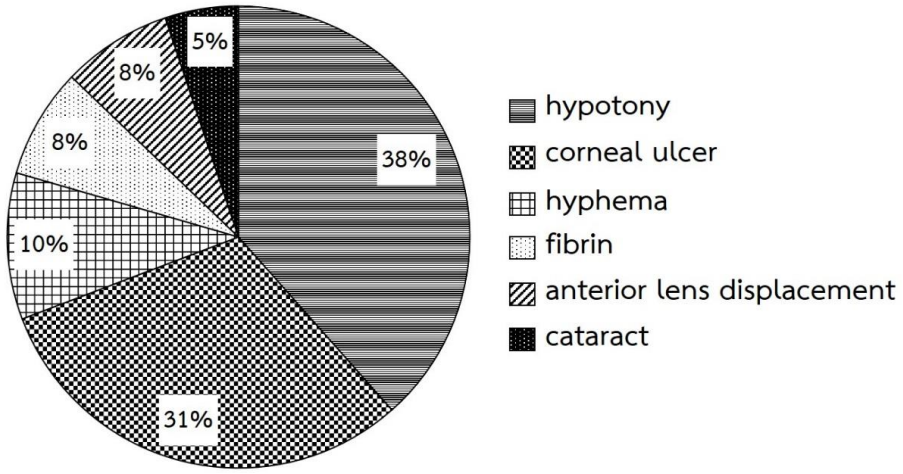

Figure 22. Schematic diagram demonstrating percentages of clinical complications post TSCP.

Hyphema was observed at approximately 1 month after TSCP (figure 23C). Volume of hyphema was ranged from one fourth to half of the anterior chamber. Dose of systemic corticosteroid was re-adjusted to control inflammation. Dog's activity was strictly minimized. Tranexamic acid (250 mg/capsule) was additionally given twice daily until accumulated blood was resolved (within a month thereafter). Three eyes developed anterior displacement of lens at day 26, 44 and 60 post TSCP. Opacity of lens was observed in two eyes at day 80 following TSCP. Most of the eyes had constricted pupils, therefore cloudiness of lens could not be well observed.

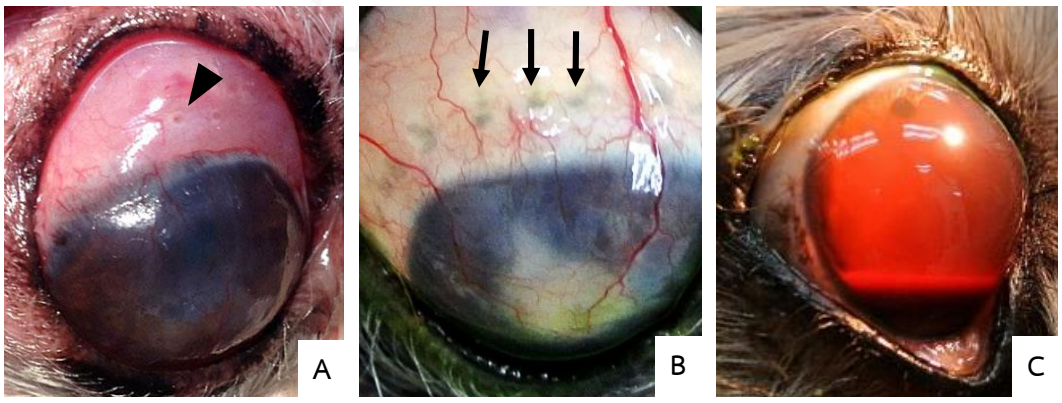

Figure 23. Photographs of complications.

A) Scleral perforation following TSCP (arrowhead); B) Pinpoint black spot (arrow); C) Hyphema.

Hypotony was noted in 15 eyes indicated by persistent low IOP. None of eyes developed intraocular infection. Vitreous hemorrhage and retinal detachment were not observed by ocular ultrasonography at day 105 post TSCP.

Part 3. Pathological study of pars plicata following TSCP

Gross examination of absolute glaucomatous eyes revealed clear demarcated pars plicata, located between the iris and the pars plana. Histologically, canine pars plicata was characterized by ciliary processes, which consisted of approximately 75-78 radial ridges (figure 24A), attaching to ciliary stroma. Ciliary stroma is composed of ground substances, together with collagens and fibrocytes. The outermost layer of pars plicata was sclera that highly contained collagen and elastic fibers (figure 24B).

Ciliary process was covered with two layers of epithelium (figure 25A). The outer epithelial layer, which was adjacent to the ciliary stroma, was pigmented and composed of cuboidal cells. The inner layer was formed by nonpigmented columnar epithelium. This layer was located adjacent to aqueous humor in the posterior chamber. In canine glaucomatous eye, several numbers of hydropic cells were found in nonpigmented epithelial layer of pars plicata (figure 25B). Vascular congestion was observed in the inner layer of ciliary stroma.

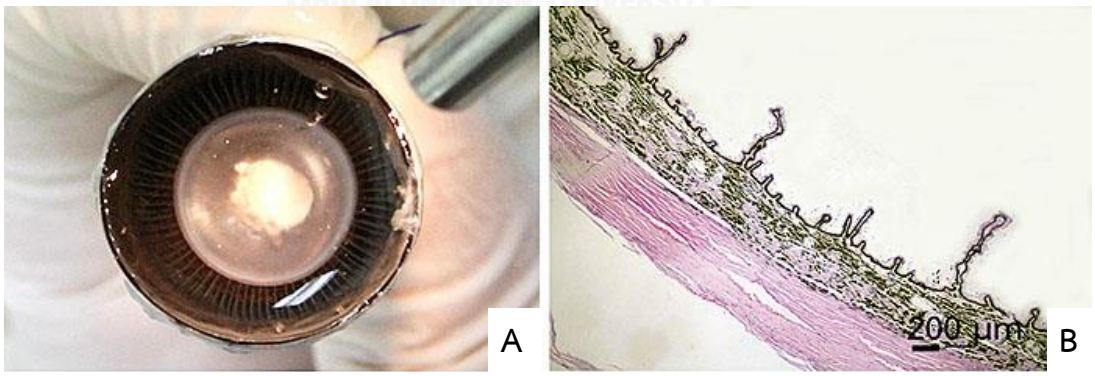

Figure 24. Photographs of canine absolute glaucomatous eye.
A) Anterior segment of the eye demonstrating radial ridges of ciliary process; B) Histology photomicrograph of pars plicata and sclera (H&E stain, bar= 200 um)

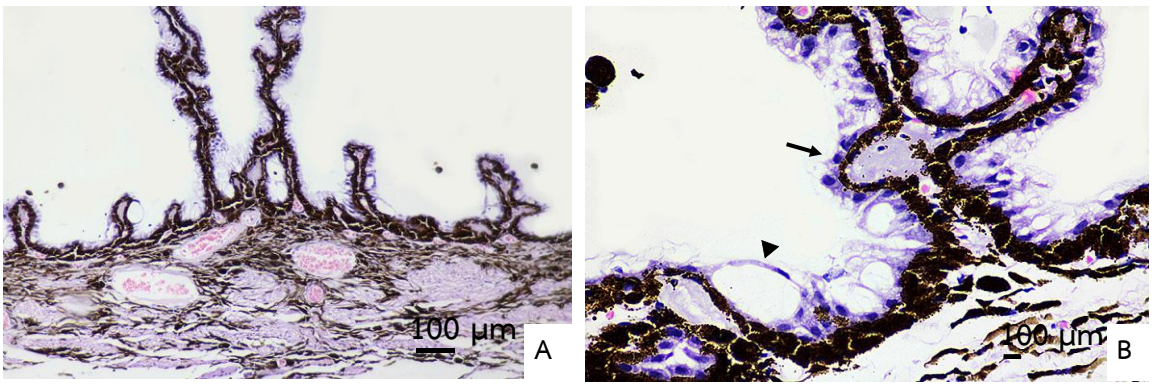

Figure 25. Histology photomicrograph of pars plicata of canine absolute glaucomatous eye.

A) Vascular congestion in the ciliary stroma (H&E stain, bar = 100 um); B) Hydropic cells were located in the inner nonpigmented layers of pars plicata (arrowshead) as compared to normal epithelial cell (arrow) (H&E stain, bar= 100 um)

Following TSCP at 32 sites to each half of the eyecup, pars plicata grossly demonstrated increased intensity of whitening of ciliary process (figure 26B). On the contrary, intensity of ciliary process on the other half of the eyecup, at which was not been exposed to diode laser, grossly appeared normal. Area at 3.00 and 9.00 positions clockwise showed same appearance as compared to non TSCP-treated regions. Multiple black spots on the sclera indicated sites of laser applications (figure 26A).

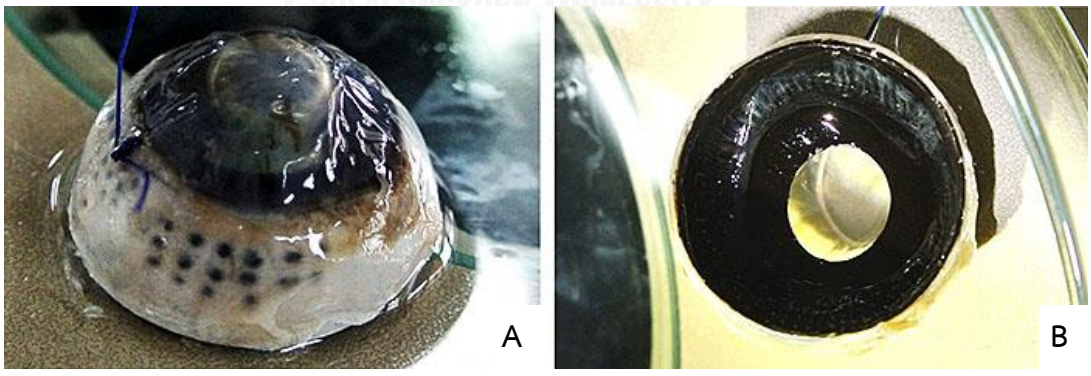

Figure 26. Photographs of TSCP-treated eye.

A) Dark spots on the sclera representing locations where laser beam was transsclerally introduced; B) Dorsal part of the eyecup representing grayish-white region of ciliary processes that were exposed to diode laser.

Microscopic examinations revealed multifocal massive denatured protein precipitated in pars plicata, ciliary stroma and partially sclera of all TSCP-treated eyes. Coagulative necrotic were as showed dense basophilic staining without inflammatory cell aggregation located along laser exposed areas (figure 27). Less intense tissue necrosis was evident at adjacent region to the laser site.

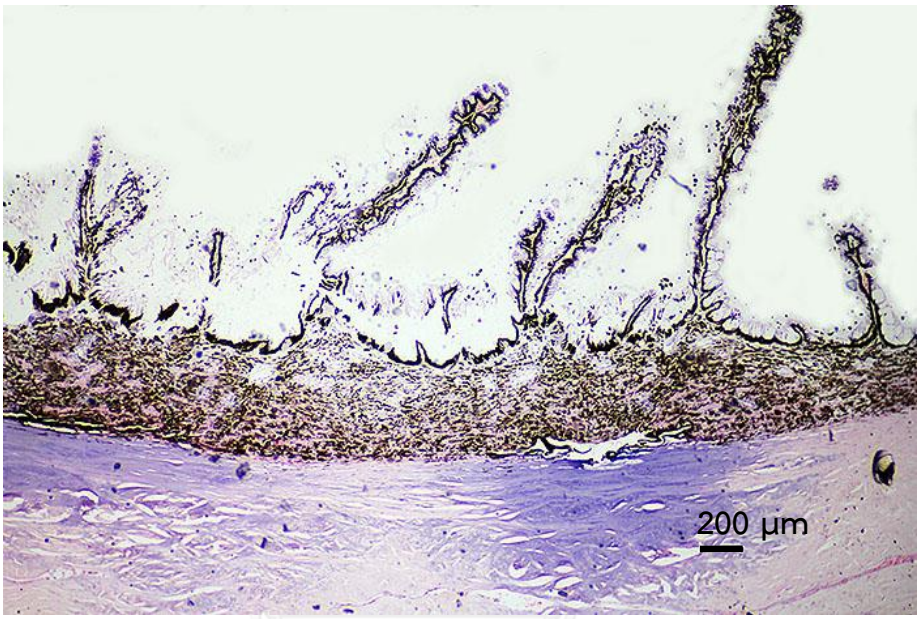

Figure 27. Histology photomicrograph of TSCP-treated region. (H&E stain, bar= 200 um) Note that there were two laser sites where sclera was changed in color.

Destruction of the ciliary processes, including both pigmented and non-pigmented epithelium was microscopically evident in TSCP-treated eyes. Non pigmented epithelium was disrupted at different degree of extent (figure 28A & 28B). There was clumping brown-black melanin pigment dispersing from the pigmented epithelium to the non-pigmented epithelium. Separation of ciliary epithelium from ciliary stroma (Figure 28B) and destruction of ciliary stroma was noted (Figure 29), coagulative necrosis also occurred at tunica adventitia as well as tunica media of vascular wall in the region of TSCP treatment (figure 30).

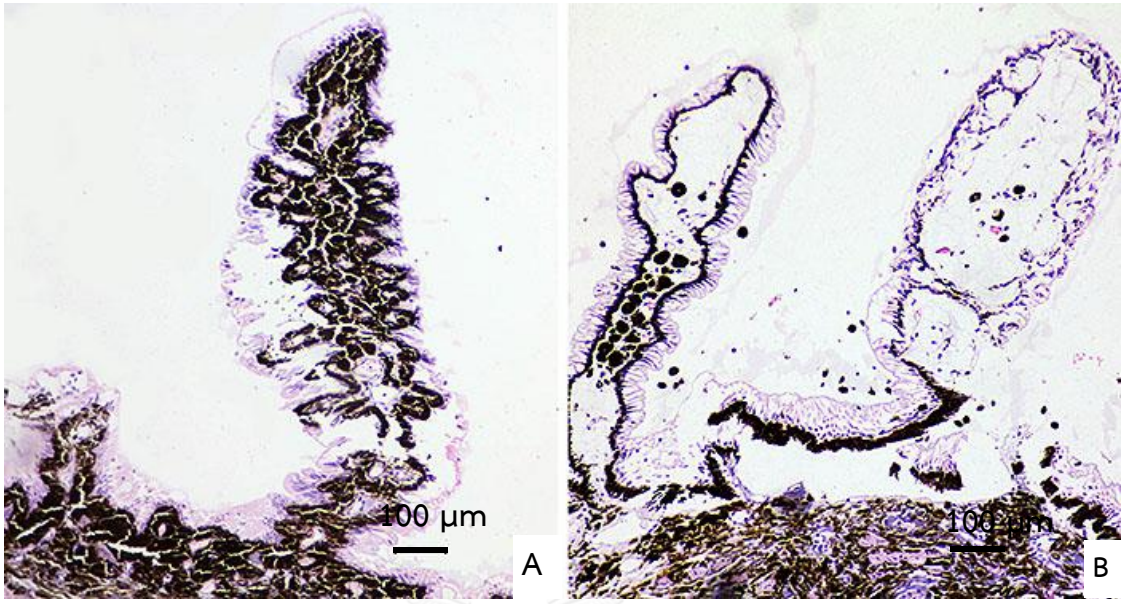

Figure 28. Histology photomicrographs of ciliary processes of TSCP-treated eye. (H&E stain, bar= 100 um).

A) Destruction of pigmented and non-pigmented ciliary epithelium lining in ciliary process; B) Ciliary epithelium was severely disrupted and separated from ciliary stroma. Heavy pigment clump was noted.

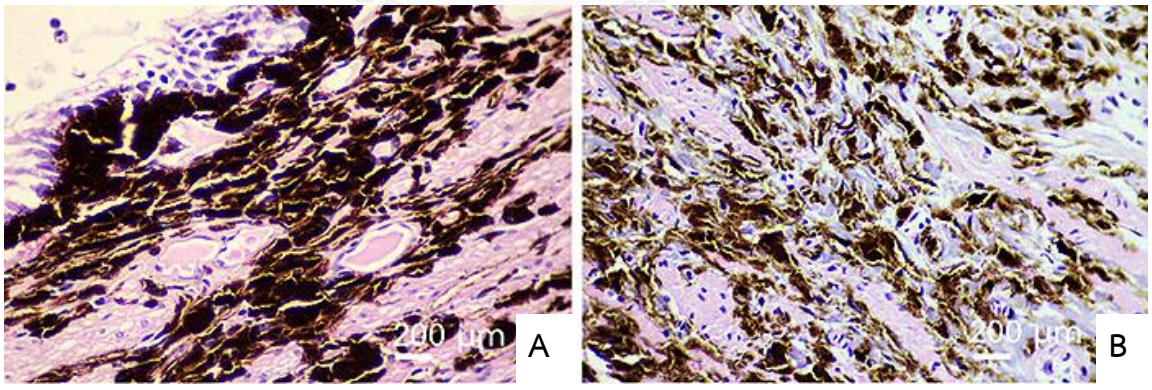

Figure 29. Histology photomicrographs of ciliary stroma. (H&E stain, bar = 200 um)

A) Ciliary stroma of non TSCP-treated eye; B) Ciliary stroma of TSCP-treated eye. Note that collagen fibers were coagulative necrosis.

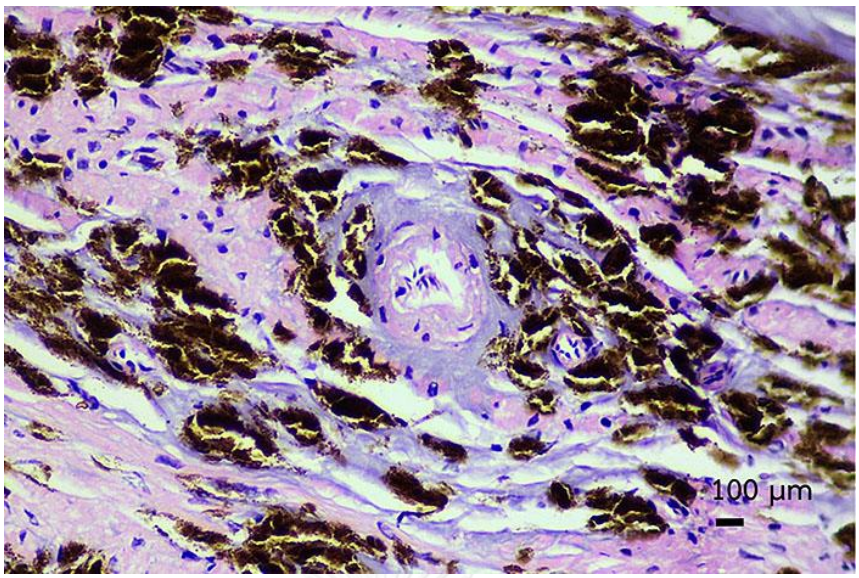

Figure 30. Histology photomicrographs of vascular wall necrosis in ciliary stroma of TSCP-treated eye. (H&E stain, bar = 100 um)

Not only architectural disruption was observed at ciliary epithelium, it was also present at ciliary stroma, resulting in focal separation of ciliary stroma from sclera (figure 31). However, hemorrhage and acute inflammatory cell infiltration were not observed in these TSCP-treated regions.

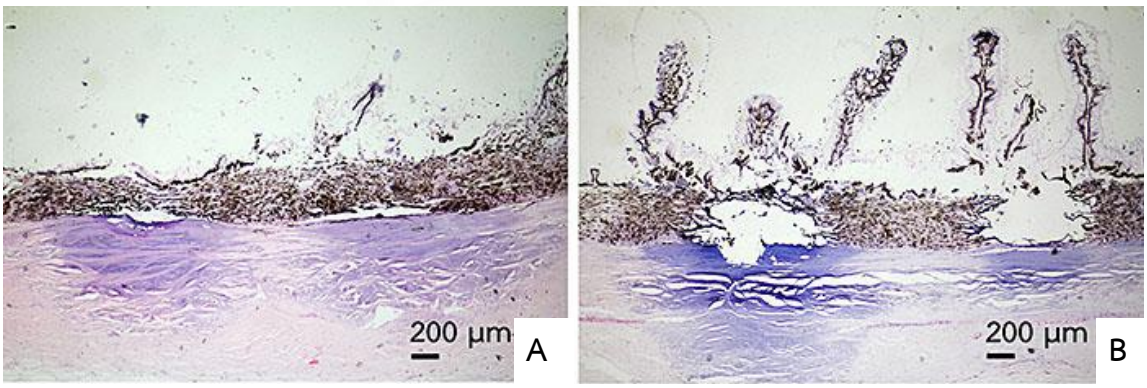

Figure 31. Histology photomicrographs of severe tissue destruction in TSCP-treated eye. (H&E stain, bar = 200 um)

A) Moderate degree of coagulative necrosis. Note that separation of ciliary stroma from sclera was observed; B) Severe degree of coagulative necrosis. Note that severe tissue disruption was evident.

Chapter 5
Discussion and Conclusion

With the use of diode laser TSCP in canine absolute glaucoma, we have shown in this study that IOP was successfully controlled (30/31) at a level below 20 mmHg throughout the study. TSCP is a procedure that the diode laser is introduced into the eye via sclera and absorbed by pigmented epithelial cells at pars plicata. Thus, coagulative necrosis occurred (Schuman et al., 1990; McKelvie and Walland, 2002; Morreale et al., 2007), as histologically demonstrated in this study. Due to the fact that, it is a noninvasive technique that does not require surgical incision, it become a universal procedure to treat glaucoma in humans (Mandal et al., 2009) and companion animals (Spiess, 2012) worldwide. To our knowledge, this is the first study using TSCP in canine absolute glaucoma with buphthalmic eye.

To achieve the great benefit in controlling IOP in glaucomatous eyes, appropriate number of ciliary epithelial cells must be destroyed following TSCP. TSCP is performed by applying a laser probe on the conjunctiva, posterior to the limbus, at which pars plicata is located. Laser beam is emitted without direct visualization of target cells. Therefore, the exact position of pars plicata should be marked externally to avoid misdirection of the laser beam (Miller et al., 2001). Ichihara et al. (2006) reported the distance from the limbus to where the posterior boundary of pars plicata is in dogs. In a buphthalmic globe, the laser probe should be applied at the distance more posterior to the limbus than in a normally sized globe (Gemensky-Metzler et al., 2014).

From this study, the nearest and the farthest boundaries of pars plicata appeared to be at the same clock wise locations as described by Ichihara et al. (2006). It was correlated to the result that axial length of the buphthalmic globe was significantly longer, compared to that of normal eyes. Width of pars plicata was therefore circumferentially elongated. Applications of the laser probe at three different distances up to 5 mm posterior to the limbus, 360 degrees around the clock, was hence sufficient to cover target cells of the laser beam. However, the locations at 3.00

and 9.00 at which a long posterior ciliary artery was located, was still avoided from being lasered (Hardman and Stanley, 2001).

Our protocol of using the laser power at 1,500 mW and exposure time of 1,500 msec resulted in sustained low IOP, reduced hypotensive drug administration and decreased axial globe length. Total laser energy of 2.25 joule per site was delivered at 64 application spots. In humans with end-stage glaucoma, the same setting of the laser energy could not only maintain desirable IOP for 10 months (Walland, 1998), but also preserve normal architecture of ciliary processes (Morreale et al., 2007). This setting protocol of power-to-duration of exposure was also recommended to be used in veterinary practice by Spiess (2012). Even though the increase in total amount of energy delivered per eye may yield an increase in rate of success (Hauber and Scherer, 2002), the high total energy could cause severe damage to ciliary process, thus creating severe inflammation post TSCP (Ueda et al., 2000).

Following TSCP, acute elevation of the IOP occurred within several days, as it was reported in canine primary glaucoma (Hardman and Stanley, 2001). We speculate that it is according to the effect of photothermal consequence in laser-tissue interaction. After transmission of laser light into target tissues, photons impart great energy to the target cells. Subsequent conversion of light energy to diffusing heat through tissues gives rise in temperature in target cells and surrounding areas. Due to the fact that laser light is coherent, collimated and intense (Berger and Eeg, 2006) spontaneous emission of photons giving rise to accumulated heat causes an abrupt increase in the IOP. When tissue temperature was above 100 C, cell disruption was indicated as “audible” pop sound (Morreale et al., 2007). Although pop sound had failed to show the correlation of its presence and therapeutic response (Rebolleda et al., 1999), our comparably high percentage of pop sound may somewhat be associated with excessive tissue damage; separations among pars plicata components and some degrees of architectural destruction. We speculated that marked thinning of sclera in buphthalmic eyeball that resulted in dark burn at sites of TSCP, may play a role in high temperature at ciliary epithelium. Variation of anatomical structure and pigmentation of the ciliary body may be taken into account in a success of cyclophotocoagulation.

One of the cases, of which the IOP had reduced but not yet reached 20 mmHg at the end of the study, had color-diluted coat. Although destruction was found in both pigmented and non-pigmented ciliary epithelium in laser-treated areas (Pantcheva et al., 2007), level of pigmentation of ciliary body is important to obtain good quality response of TSCP (Cantor et al., 1989). TSCP with neodymium-YAG laser in albino rabbits revealed no evidence of disruption of pigmented or non-pigmented ciliary epithelium or vascular network. In the meantime, extensive destruction of stromal melanocytes and inflammation were evident in pigmented rabbit eyes.

Degree of lowering the IOP with this particular laser setting protocol should be efficient to create strong coagulation at the ciliary process. Vicious destruction caused by inflammatory product then results in marked reduction of aqueous humor production (Ueda et al., 2000), leading to reduction of the axial globe length. Furthermore, shrinkage of cliary stroma histologically identified with presence of fibrosis in the ciliary stroma of pigmented rabbits at 24 weeks after TSCP (Ueda et al., 2000) may additionally play a role in a decrease in size of TSCP-treated eyeballs. To avoid hypotony that had developed after intense coagulation, re-adjustment of the laser setting protocol during TSCP procedure may be considered. In theory, the setting protocol that reached optimum total energy per site; but yet maintain temperature of cellular tissue at 60-100 °C for coagulative necrosis (Berger and Eeg, 2006) was recommended. High success in IOP control by using high power protocol was demonstrated by Noureddin et al. (2005). For this reason, application of the low power setting protocol with lesser number of pop sounds may result in less success of IOP control as compared to the high laser power protocol with high number of pop sounds. Among different quadrants of a globe, percentage of pop sound was statistically comparable. However, it was statistically significant lower at 5 mm compared to that at 3 and 4 mm posterior to the limbus. Reduction of laser application at 5 mm posterior to the limbus at the nasal region may reduce degree of tissue injury.

Following TSCP, there were no apparent clinical signs related to ocular discomfort. By time, eyes were no longer buphthalmic. Ulcerative keratitis developing after TSCP was common (Whigham et al., 1999). Damage of the cornea may be related

to laser the energy introduced close to the limbus (Johnson, 1998). A decrease in corneal sensitivity from long-term elevation of IOP in chronic glaucoma was hypothesized (Hardman and Stanley, 2001), although Raivio et al. (2002) reported non-impaired corneal innervations. Temporal tarsorrhaphy caused irritation in some dogs. This may be accounted as a cause of corneal ulcer at the beginning of our study.

Hyphema was reported as one of major clinical complications post TSCP (Annear et al., 2010). Pathogenesis of hyphema involves dysfunction of blood-aqueous-barrier. Breakdown of blood-aqueous-barrier is associated with Inflammation of the uvea caused by cyclophotocoagulation. In normal ocular condition, IOP that is higher than pressure within the aqueous drainage pathway or sclerovenous plexus prevents retrograde blood flow into the anterior chamber (Komáromy et al., 1999). Retrograde blood flow may have occurred in one of the dogs developing hyphema immediately after anterior chamber paracentesis. Since chronic uveal inflammation was pathologically evident in TSCP-treated eyes (Annear et al., 2010), pre-iridal fibrovascular membranes (PFIM) can possibly occur (Zarfoss et al., 2010) as a consequence. PFIM is mediated by angiogenic and fibroblastic stimulatory factors from chronic inflammation (Peiffer et al., 1990). It is a membrane extending from iridal stroma onto the anterior surface of the iris. PFIM contains vessels, extracellular matrix and inflammatory cells. With newly-formed vessels, it is fragile and potentially resulting in hemorrhage. Therefore it was not unusual that hyphema developed in four eyes at approximately one month after TSCP.

There were two eyes developing cataract which might be related to metabolic alterations of aqueous humor in maintaining normal integrity of lens (Ofri, 2013). As hypotony developed toward the end of our study, cataract and rupture of lens zonule were more likely to develop subsequently.

Conclusion

Transscleral diode laser cyclophotocoagulation in canine absolute glaucoma demonstrated a significant success in permanently lowering IOP, reducing axial globe length and minimizing the use of topical hypotensive medications. Ulcerative keratitis

and hyphema were the main clinical complications that resolved after treatment. Dogs received better quality of life from no ocular pain and exposure keratitis. As a result of requiring less/no eye drops for glaucoma treatment, medical expense was extraordinarily decreased.

Suggestions

Laser setting protocol should appropriately be adjusted in treating various types of canine glaucoma. In a bupthalmic globe, laser application at 5 mm posterior to the limbus at nasoventral region where the pars plicata width was shortest may be unnecessary. Temporary tarsorrhaphy is not recommended following TSCP to avoid development of corneal ulcer. In the visual eye, a protocol of less power with longer duration of exposure may be considered.

REFERENCES

Abrams KL. 2001. Medical and surgical management of the glaucoma patient. Clin Tech Small Anim Pract. 16(1): 71-76.

Alward WLM. 1998. Medical Management of Glaucoma. N Engl J Med. 339(18): 1298-1307.

Ammar DA, Noecker RJ and Kahook MY. 2010. Effects of benzalkonium chloride-preserved, polyquad-preserved, and sofZia-preserved topical glaucoma medications on human ocular epithelial cells. Adv Ther. 27(11): 837-845.

Andrade SF, Palozzi RJ, Giuffrida R, de Campos RJ, de Campos Santos G and Fukui RM. 2012. Comparison of intraocular pressure measurements between the Tono-Pen XL® and Perkins® applanation tonometers in dogs and cats. Vet Ophthalmol. 15: 14-20.

Annear MJ, Wilkie DA and Gemensky-Metzler AJ. 2010. Semiconductor diode laser transscleral cyclophotocoagulation for the treatment of glaucoma in horses: a retrospective study of 42 eyes. Vet Ophthalmol. 13(3): 204-209.

Beckman H, Kinoshita A, Rota A and Sugar H. 1972. Transscleral ruby laser irradiation of the ciliary body in the treatment of intractable glaucoma. Trans Sect Ophthalmol Am Acad Ophthalmol Otolaryngol. 76(2): 423-436.

Berger N and Eeg PH. 2006. Theory of Laser Surgery. In: Veterinary Laser Surgery: A Practical Guide. ed. N Berger and PH Eeg (ed.). Lowa: Blackwell Publishing. 1-76.

Bill A and WåLINDER P-E. 1966. The effects of pilocarpine on the dynamics of aqueous humor in a primate (Macaca irus). Invest Ophthalmol Vis Sci. 5(2): 170-175.

Cantor L, Nichols D, Katz L, Moster M, Poryzees E, Shields J and Spaeth G. 1989. Neodymium-YAG transscleral cyclophotocoagulation. The role of pigmentation. Invest. Ophthalmol. Vis. Sci. 30(8): 1834-1837.

Cook C, Davidson M, Brinkmann M, Priehs D, Abrams K and Nasisse M. 1997. Diode laser transscleral cyclophotocoagulation for the treatment of glaucoma in dogs: results of six and twelve month follow-up (abstract). Vet Comp Ophthalmol 7: 148-154.

Egbert PR, Fiadoyor S, Budenz DL, Dadzie P and Byrd S. 2001. Diode laser transscleral cyclophotocoagulation as a primary surgical treatment for primary open-angle glaucoma. Arch Ophthalmol. 119(3): 345-350.

Fea A, Damato D, Lorenzi U and Grignolo FM. 2011. Another Look on Cyclodestructive Procedures In: Glaucoma - Basic and Clinical Concepts. ed. R. Shimon (ed.). Shanghai: InTech. 451-468.

Fiscella RG, Green A, Patuszynski DH and Wilensky J. 2003. Medical therapy cost considerations for glaucoma. Am J Ophthalmol. 136(1): 18-25.

Frishman WH, Fuksbrumer MS and Tannenbaum M. 1994. Topical ophthalmic β-adrenergic blockade for the treatment of glaucoma and ocular hypertension. J Clin Pharmacol. 34(8): 795-803.

Gelatt KN, Brooks DE and Kallberg ME. 2007. The Canine Glaucomas In: Veterinary Ophthalmology. 4th ed. KN Gelatt (ed.). Lowa: Blackwell Publishing. 753-811.

Gelatt KN, Esson DW and Plummer CE. 2011. Surgical procedures for the glaucomas. In: Veterinary Ophthalmic Surgery. ed. KN Gelatt and JP Gelatt (ed.). Oxford: Elsevier. 263-303.

Gelatt KN and MacKay EO. 2001. Effect of different dose schedules of latanoprost on intraocular pressure and pupil size in the glaucomatous Beagle. Vet Ophthalmol. 4(4): 283-288.

Gelatt KN and MacKay EO. 2004a. Prevalence of the breed-related glaucomas in pure-bred dogs in North America. Vet Ophthalmol. 7(2): 97-111.

Gelatt KN and MacKay EO. 2004b. Secondary glaucomas in the dog in North America. Vet Ophthalmol. 7(4): 245-259.

Gemensky-Metzler AJ, Wilkie DA, Weisbrode SE and Kuhn SE. 2014. The location of sites and effect of semiconductor diode trans-scleral cyclophotocoagulation on the buphthalmic equine globe. Vet Ophthalmol. 17: 107-116.

Goel M, Picciani RG, Lee RK and Bhattacharya SK. 2010. Aqueous humor dynamics: a review. Open Ophthalmol J. 4: 52.

Hardman C and Stanley RG. 2001. Diode laser transscleral cyclophotocoagulation for the treatment of primary glaucoma in 18 dogs: A retrospective study. Vet Ophthalmol. 4(3): 209-215.

Hauber FA and Scherer WJ. 2002. Influence of total energy delivery on success rate after contact diode laser transscleral cyclophotocoagulation: A retrospective case review and meta-analysis. J Glaucoma. 11(4): 329-333.

Hong C-H, Arosemena A, Zurakowski D and Ayyala RS. 2005. Glaucoma drainage devices: A systematic literature review and current controversies. Surv Ophthalmol. 50(1): 48-60.

Ichihara N, Kikuchi N, Sawada T, Kaseda M, Honnma N, Shibata S, Amasaki H and Asari M. 2006. Macromorphometric study on ciliary body location in canine eyes. J Vet Med Sci. 68(3): 201-204.

Infeld DA and O'Shea JG. 1998. Glaucoma: Diagnosis and management. Postgrad Med J. 74(878): 709-715.

Johnson SM. 1998. Neurotrophic corneal defects after diode laser cycloablation. Am J Ophthalmol. 126(5): 725-727.

Kim DD and Moster MR. 1999. Transpupillary argon laser cyclophotocoagulation in the treatment of traumatic glaucoma (abstract). J Glaucoma. 8(5): 340-341.

Kitazawa Y, Kawase K, Matsushita H and Minobe M. 1991. Trabeculectomy with mitomycin: A comparative study with fluorouracil. Arch Ophthalmol. 109(12): 1693-1698.

Knollinger AM, La Croix NC, Barrett PM and Miller PE. 2005. Evaluation of a rebound tonometer for measuring intraocular pressure in dogs and horses. J Am Vet Med Assoc. 227(2): 244-248.

Komáromy AM, Ramsey DT, Brooks DE, Ramsey CC, Kallberg ME and Andrew SE. 1999. Hyphema. Part I. Pathophysiologic considerations. Comp Cont Educ Pract. 21(11): 1064-1069.

Kramp K, Vick H-P and Guthoff R. 2002. Transscleral diode laser contact cyclophotocoagulation in the treatment of different glaucomas, also as primary surgery. Graefe's Arch Clin Exp Ophthalmol. 240(9): 698-703.

Lee DA and Higginbotham EJ. 2005. Glaucoma and its treatment: A review. Am J Health Syst Pharm. 62(7): 691-699.

Leiva M, Naranjo C and Peña MT. 2006. Comparison of the rebound tonometer (ICare®) to the applanation tonometer (Tonopen XL®) in normotensive dogs. Vet Ophthalmol. 9(1): 17-21.

Lew M and Lew S. 2007. Cyclodialysis in the treatment of nonhealing secondary glaucoma. Pol J Vet Sci. 11(4): 371-375.

Lew M and Lew S. 2009. Combined iridencleisis and posterior sclerectomy in surgical treatment of glaucoma secondary to uveitis in a dog: A case report. Vet Med 54(3): 142-148.

Lin C-T, Hu C-K, Liu C-H and Yeh L-S. 2007. Surgical outcome and ocular complications of evisceration and intraocular prosthesis implantation in dogs with end stage glaucoma: a review of 20 cases. J Vet Med Sci. 69(8): 847-850.

Lin P, Wollstein G and Schuman JS. 2004. Contact transscleral neodymium:yttrium–aluminum–garnet laser cyclophotocoagulation: Long-term outcome. Ophthalmol. 111(11): 2137-2143.

Lin S, Chen M, Lin M, Howes E and Stamper R. 2006. Vascular effects on ciliary tissue from endoscopic versus trans-scleral cyclophotocoagulation. Br J Ophthalmol. 90(4): 496-500.

Lin SC. 2008. Endoscopic and transscleral cyclophotocoagulation for the treatment of refractory glaucoma. J. Glaucoma. 17(3): 238-247.

Lorimer D, Hakanson N, Pion P and Merideth R. 1989. The effect of intravenous mannitol or oral glycerol on intraocular pressure in dogs. Cornell Vet. 79(3): 249-258.

Low MC, Landis ML and Peiffer RL. 2014. Intravitreal cidofovir injection for the management of chronic glaucoma in dogs. Vet ophthalmol. 17(3): 201-206.

Mahmood K, Khan MT, Butt JBY and Qureshi T. 2011. Diode laser trans–scleral cyclo–ablation as a primary surgical treatment for primary open–angle glaucoma after maximum tolerated medical therapy. Annals 17(2): 157-161.

Mandal S, Gadia R and Ashar J. 2009. Diode Laser Cyclophotocoagulation. JOCGP. 3(2): 47-59.

Martín-Suárez E, Molleda C, Tardón R, Galán A, Gallardo J and Molleda J. 2014. Diurnal variations of central corneal thickness and intraocular pressure in dogs from 8: 00 am to 8: 00 pm. Can Vet J. 55(4): 361.

Martinez-de-la-Casa JM, Garcia-Feijoo J, Castillo A and Garcia-Sanchez J. 2005. Reproducibility and clinical evaluation of rebound tonometry. Invest Ophthalmol Vis Sci. 46(12): 4578-4580.

McKelvie P and Walland M. 2002. Pathology of cyclodiode laser: A series of nine enucleated eyes. Br J Ophthalmol. 86(4): 381-386.

Miller PE. 2008. The glaucomas. In: Slatter's Fundamentals of Veterinary Ophthalmology. 4th ed. DJ Maggs, PE Miller, and R Ofri (ed.). Missouri: Saunders. 247-271.

Miller TL, Willis AM, Wilkie DA, Hoshaw-Woodard S and Stanley JRL. 2001. Description of ciliary body anatomy and identification of sites for transscleral cyclophotocoagulation in the equine eye. Vet Ophthalmol. 4(3): 183-190.

Morreale RJ, Wilkie DA, Gemensky-Metzler AJ, Weisbrode SE and Willis MA. 2007. Histologic effect of semiconductor diode laser transscleral cyclophotocoagulation on the normal equine eye. Vet Ophthalmol. 10(2): 84-92.

Nadelstein B, Wilcock B, Cook C and Davidson M. 1997. Clinical and histopathologic effects of diode laser transscleral cyclophotocoagulation in the normal canine eye (abstract) Vet Comp Ophthalmol. 7: 155-162.

Noureddin B, Zein W, Haddad C, Ma'luf R and Bashshur Z. 2005. Diode laser transcleral cyclophotocoagulation for refractory glaucoma: a 1 year follow-up of patients treated using an aggressive protocol. Eye. 20(3): 329-335.

Ofri R. 2013. lens. In: Slatter's Fundamentals of Veterinary Ophthalmology. 5th ed. DJ Maggs, PE Miller, and R Ofri (ed.). Missouri: Elsevier. 272-290.

Pantcheva MB, Kahook MY, Schuman JS and Noecker RJ. 2007. Comparison of acute structural and histopathological changes in human autopsy eyes after endoscopic cyclophotocoagulation and trans-scleral cyclophotocoagulation. Br J Ophthalmol. 91(2): 248-252.

Park Y-W, Jeong M-B, Kim T-H, Ahn J-S, Ahn J-T, Park S-A, Kim S-E and Seo K. 2011. Effect of central corneal thickness on intraocular pressure with the rebound tonometer and the applanation tonometer in normal dogs. Vet Ophthalmol. 14(3): 169-173.

Pastor SA, Singh K, Lee DA, Juzych MS, Lin SC, Netland PA and Nguyen NT. 2001. Cyclophotocoagulation: a report by the American Academy of Ophthalmology. Ophthalmol. 108(11): 2130-2138.

Patel A, Thompson JT, Michels RG and Quigley HA. 1986. Endolaser treatment of the ciliary body for uncontrolled glaucoma (abstract). Ophthalmol. 93(6): 825-830.

Peiffer R, Wilcock B and Yin H. 1990. The pathogenesis and significance of pre-iridal fibrovascular membrane in domestic animals. Veterinary Pathology Online. 27(1): 41-45.

Playfair TJ and Watson PG. 1979. Management of acute primary angle-closure glaucoma: a long-term follow-up of the results of peripheral iridectomy used as an initial procedure. Br J Ophthalmol. 63(1): 17-22.

Pucci V, Tappainer F, Borin S and Bellucci R. 2003. Long-term follow-up after transscleral diode laser photocoagulation in refractory glaucoma. Ophthalmologica. 217(4): 279-283.

Quigley HA and Broman AT. 2006. The number of people with glaucoma worldwide in 2010 and 2020. Br J Ophthalmol. 90(3): 262-267.

Raivio VE, Immonen IJR and Puska PM. 2001. Transscleral contact krypton laser cyclophotocoagulation for treatment of glaucoma in children and young adults1. Ophthalmol. 108(10): 1801-1807.

Raivio VE, Vesaluoma MH, Tervo TM, Immonen IJ and Puska PM. 2002. Corneal innervation, corneal mechanical sensitivity, and tear fluid secretion after transscleral contact 670-nm diode laser cyclophotocoagulation. J. glaucoma. 11(5): 446-453.

Rebolleda G, Muñoz FJ and Murube J. 1999. Audible Pops During Cyclodiode Procedures. J Glaucoma. 8(3): 177-183.

Reinstein SL, Rankin A and Allbaugh R. 2009a. Canine Glaucoma: Medical and Surgical Treatment Options. Compendium 31(10): 454-458.

Reinstein SL, Rankin AJ and Allbaugh R. 2009b. Canine glaucoma: Pathophysiology and diagnosis. Compendium. 31(10): 450-452.

Renwick P. 2002. Glaucoma. In: BSAVA Manual of Small Animal Ophthalmology. 2nd ed. S Petersen-Jones and S Crispin (ed.). Gloucester: British Small Animal Veterinary Association. 185-203.

Sapienza JS. 2008. Surgical procedures for glaucoma: what the general practitioner needs to know. Top Companion Anim Med. 23(1): 38-45.

Schuman JS, Jacobson JJ, Puliafito CA, Noecker RJ and Reidy WT. 1990. Experimental use of semiconductor diode laser in contact transscleral cyclophotocoagulation in rabbits. Arch Ophthalmol. 108(8): 1152-1157.

Scott EM and McLellan GJ. 2013. Top 5 Glaucoma drugs. Clinician's brief. 81-83.

Sha'aban RI and Asfour WM. 2004. Efficacy of Contact Trans-Scleral Diode Laser Cyclophotocoagulation For Neovascular Glaucoma. J Bahrain Med Soc. 16(3): 123-127.

Shields MB. 1985. Cyclodestructive surgery for glaucoma: past, present, and future. Trans Am Ophthalmol Soc. 83: 285-303.

Skorobohach BJ, Ward DA and Hendrix DV. 2003. Effects of oral administration of methazolamide on intraocular pressure and aqueous humor flow rate in clinically normal dogs. Am J Vet Res. 64(2): 183-187.

Spiess BM. 2012. The use of lasers in veterinary ophthalmology: Recommendations based on literature. Photonics Lasers Med. 1(2): 95-102.

Steinmetz A and Oechtering G. 2008. Management of glaucoma in the dog and cat. Tierärztl Prax 36: 63-66.

Strom AR, Hässig M, Iburg TM and Spiess BM. 2011a. Epidemiology of canine glaucoma presented to University of Zurich from 1995 to 2009. Part 1: Congenital and primary glaucoma (4 and 123 cases). Vet Ophthalmol. 14(2): 121-126.

Strom AR, Hässig M, Iburg TM and Spiess BM. 2011b. Epidemiology of canine glaucoma presented to University of Zurich from 1995 to 2009. Part 2: Secondary glaucoma (217 cases). Vet Ophthalmol. 14(2): 127-132.

Tham Y-C, Li X, Wong TY, Quigley HA, Aung T and Cheng C-Y. 2014. Global prevalence of glaucoma and projections of glaucoma burden through 2040: A systematic review and meta-analysis. Ophthalmol. 121(11): 2081-2090.

To C-h, Kong C-w, Chan C-y, Shahidullah M and Do C-w. 2002. The mechanism of aqueous humour formation. Clin Exp Optom. 85(6): 335-349.

Toris CB, Camras CB and Yablonski ME. 1999. Acute versus chronic effects of brimonidine on aqueous humor dynamics in ocular hypertensive patients. Am J Vet Res. 128(1): 8-14.

Toris CB, Gleason ML, Camras CB and Yablonski ME. 1995. Effects of brimonidine on aqueous humor dynamics in human eyes. Arch Ophthalmol. 113(12): 1514-1517.

Ueda N, Obana A and Miki T. 2000. Treatment parameters for the efficacy of transscleral cyclophotocoagulation in rabbits using a diode laser. Jpn J Ophthalmol. 44(3): 205-213.

Walland MJ. 1998. Diode laser cyclophotocoagulation: Dose‐standardized therapy in end‐stage glaucoma*. Aust N Z J Ophthalmol. 26(2): 135-139.

Whigham HM, Brooks DE, Andrew SE, Gelatt KN, Strubbe and Biros DJ. 1999. Treatment of equine glaucoma by transscleral neodymium:yttrium aluminum garnet laser cyclophotocoagulation: a retrospective study of 23 eyes of 16 horses. Vet Ophthalmol. 2(4): 243-250.

Youn J, Cox TA, Herndon LW, Allingham R and Shields MB. 1998. A clinical comparison of transscleral cyclophotocoagulation with neodymium: YAG and semiconductor diode lasers. Am J Ophthalmol. 126(5): 640-647.

Zarfoss MK, Breaux CB, Whiteley HE, Hamor RE, Flaws JA, Labelle P and Dubielzig RR. 2010. Canine pre‐iridal fibrovascular membranes: morphologic and immunohistochemical investigations. Veterinary ophthalmology. 13(1): 4-13.

APPENDIX

VITA

Miss Pataraporn Winayanuwattikhun was born on July 25, 1987 in Bangkok, Thailand. She received her bachelor of Doctor of Veterinary Medicine (D.V.M.); second honor, from the Faculty of Veterinary Science, Chulalongkorn University, Thailand, in 2010. After graduation, she had spent a year as a clinician in private small animal hospital. In 2012, she entered the Master's Degree program in Veterinary Surgery of Chulalongkorn University.