A DISSERTATION

FOR THE DEGREE OF DOCTOR OF PHILOSOPHY

Analysis of Anterior Ocular Segment Using Ultrasound Biomicroscopy in Normal Pigeons and Topical Anti-Glaucoma Drug Treated Dogs

정상 비둘기와 녹내장 안약을 투여한 개에서 초음파 생체현미경을 이용한 전안부 분석

by

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Analysis of Anterior Ocular Segment Using Ultrasound Biomicroscopy in Normal Pigeons and Topical Anti-Glaucoma Drug Treated Dogs

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ABSTRACT

The purpose of the present study was to analyze anterior ocular segments of pigeons and dogs using ultrasound biomicroscopy (UBM).

In Chapter I, the feasibility of UBM was evaluated in pigeons and normal biometric dimensions of anterior ocular segment of pigeons were established. UBM was performed in 10 pigeons (*Columba livia* var. *domestica*). On each obtained image, the ciliary cleft (CC) length, CC width, CC area, and iridocorneal

angle (ICA) were measured. The UBM scanning procedure was well tolerated in all pigeons. Mean ± standard deviation values of CC length, CC width, CC area, and ICA were 1.55 ± 0.17 mm, 0.36 ± 0.05 mm, 0.39 ± 0.04 mm^2, and 15.17 ± 1.06°, respectively.

In Chapter II, to demonstrate the mechanism of action of topical latanoprost in dogs, the effects of topical latanoprost on the anterior segment and ciliary body were evaluated. UBM scans were performed before and 2 hours after topical latanoprost instillation. From the next day on, latanoprost was topically applied twice daily for 7 days. After 1 week of instillation, the UBM scans were repeated. The ciliary body thicknesses (CBT) as well as the anterior segment parameters, including the ICA, CC entry width, CC length, and mid-CC width were measured. The topical latanoprost decreased the ICA and CC entry width and increased the mid-CC width without any significant alterations in CC length. There were time-dependent alterations in CBT: a reduction in CBT after 2 hours of instillation and rebound thickening after 1 week of instillation. This might be a reflection of the mechanism of the uveoscleral outflow enhancement induced by the topical latanoprost.

In Chapter III, to assess the role of miosis in reducing intraocular pressure (IOP), different classes of topical miotics were selected including latanoprost which induces ciliary muscle relaxation and prostaglandin-mediated miosis and pilocarpine which induces ciliary muscle contraction and cholinergic-mediated miosis. The CC morphology was compared between prostaglandin-mediated

miosis and cholinergic-mediated miosis using UBM. Despite the similar degree of miosis, the posterior width of CC (CCW) was varied according to the contractility status of ciliary muscle and cholinergic mediated miosis caused significantly wider CCW than prostaglandin-mediated miosis. When both miotics were used in combination, the order of administration affected ciliary muscle contractility such that the first drug determined the ciliary muscle contractility and associated cleft morphology.

In conclusion, UBM was demonstrated to be clinically feasible in evaluating anterior ocular segments in small avian species and dogs. Using UBM, uveoscleral outflow enhancement by topical latanoprost was indirectly demonstrated in dogs. In addition, prostaglandin-mediated miosis by topical latanoprost and cholinergic-mediated miosis by topical pilocarpine resulted in different CC configurations in dogs. Since aqueous outflow resistance could be influenced by the width of CC, the findings of the present study should be considered in medical managements of canine glaucoma.

Keywords: ciliary cleft, dog, latanoprost, pigeon, pilocarpine, ultrasound biomicroscopy

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CONTENTS

List of Abbreviations

ANOVA	Analysis of Variance
AOD	Angle Opening Distance
CB	Ciliary Body
CBM	Ciliary Body Musculature
CBT	Ciliary Body Thickness
CC	Ciliary Cleft
CCA	Ciliary Cleft Area
CCL	Ciliary Cleft Length
CCW	Posterior Width of Ciliary Cleft
CI	Confidence Interval
CP	Ciliary Process
ECM	Extracellular Matrix
ICA	Iridocorneal Angle
ICC	Intraclass Correlation Coefficient
IOP	Intraocular Pressure
MMP	Matrix Metalloprotease
SD	Standard Deviations
UBM	Ultrasound Biomicroscopy

GENERAL INTRODUCTION

In the late 1990s, the development of ultrasound biomicroscopy (UBM) allowed the observation of living tissue at near microscopic resolution (Pavlin & Foster, 1995). UBM is a high-frequency (50 MHz) ultrasound technique using water bath, thereby increasing tissue resolution by approximately 10 times compared with a conventional 10 MHz probe and allowing discrimination of structures as small as 50 ㎛ (Dietrich, 2013).

The high resolution of the iridocorneal angle and ciliary cleft region allows qualitative and quantitative evaluation of anterior ocular segment dimensions. In veterinary ophthalmology, quantitative UBM studies have been performed in dogs (Crumley et al., 2009; Rose et al., 2008; Bentley et al., 2005), cats (Aubin et al., 2003), experimental rabbits (Werner et al., 2006) and pigs (Bartholomew et al., 1997). Since its use was limited to mammalian species to date, this study was designed to investigate the feasibility of UBM in avian species and to provide normal biometric values of anterior ocular segment dimensions in pigeons in Chapter I.

Topical latanoprost is an effective ocular hypotensive drug commonly used for the treatment of glaucoma in humans and animals. In humans and primates, topical latanoprost is demonstrated to enhance the uveoscleral outflow, thereby reducing intraocular pressure (IOP). It has been suggested that there could be time-dependent variation in the mechanism of the ocular hypotensive effect of

latanoprost (Plummer et al., 2013; Alm and Nilsson, 2009). The early effect of latanoprost on the uveoscleral outflow is considered to be mediated by a relaxation of the ciliary muscle, and the long-term effect is likely to be mediated by matrix metalloproteinase-mediated dissolution of the extracellular matrix around the ciliary muscle bundles (Alm and Nilsson, 2009). Both the ciliary muscle relaxation and the remodeling of the interstitial spaces of the ciliary musculature could contribute to the widening of the spaces around the muscle bundles and resultant increased uveoscleral outflow.

Although topical latanoprost is one of the most potent anti-glaucoma drugs in canine ophthalmology, however, the ocular hypotensive mechanism is still unclear in dogs. With the help of UBM, Chapter II was designed to elucidate the causal relationship between uveoscleral outflow and topical latanoprost.

Apart from the possibility of uveoscleral outflow enhancement, one clinical canine UBM study suggested that the rapid and potent IOP decrease of topical latanoprost could be a result of reduced iridolenticular contact at pupillary margin by latanoprost-induced miosis (Miller et al., 2003). In contrast to effects in humans and other primates, prostaglandin-mediated miosis is commonly described in dogs, cats, and horses (Plummer et al., 2013). Although not as profound as latanoprost in reducing IOP, cholinergic miotics (eg. pilocarpine) have been used for the long-term treatment of dogs with open-angle glaucoma (Plummer et al., 2013). During cholinergic-mediated miosis, the iris reportedly pulls the inner leaflet of the ciliary body rostrally and centrally, which results in widening of the CC (Carreras et al.,

1997). However, few studies have been conducted to investigate differences between prostaglandin-mediated and cholinergic-mediated miosis. Therefore, topical latanoprost was compared to topical pilocarpine in Chapter III to elucidate the role of miosis in reducing IOP.

CHAPTER I

Ultrasound Biomicroscopy and Tonometry in Ophthalmologically Normal Pigeon Eyes

Abstract

To evaluate the feasibility of ultrasound biomicroscopy (UBM) and tonometry in pigeons and to provide biometric reference ranges for normal pigeon eyes, 10 pigeons (*Columba livia* var. *domestica*) with ophthalmologically normal eyes were used. Ophthalmic examinations, including slit lamp biomicroscopy and tonometry, were performed to confirm that the eyes were normal. UBM was then performed on the left eye. On each obtained image, the ciliary cleft (CC) length, CC width, and CC area, and iridocorneal angle (ICA) were measured. Richly vascularized iris was observed in all pigeon eyes. Mean intraocular pressure was 11.7 ± 1.6 mmHg, without any statistical difference between the left and right eyes. The UBM scanning procedure was well tolerated in all pigeons. Mean values of CC length, CC width, CC area, and ICA were 1.55 ± 0.17 mm, 0.36 ± 0.05 mm, 0.39 ± 0.04 mm^2, and 15.17 ± 1.06°, respectively. UBM could be a useful diagnostic tool to evaluate anterior ocular segment of pigeons.

Introduction

Pigeons in the order Columbiformes account for almost half of all birds in captivity (Harlin and Wade, 2009). They are widely utilized for hobby, sport, entertainment, communication, and research (Harlin and Wade, 2009). Despite their long-standing popularity, ophthalmic studies of pigeons have rarely been reported.

Several studies have described the radiographic anatomy of the orbit and ultrasonographic and computed tomographic appearance of eyes in birds (Gumpenberger and Kolm, 2006; Amber et al., 2012; Lehmkuhl et al. 2010) and B-mode ultrasound has been demonstrated to be a clinically effective and rapid diagnostic tool when ophthalmoscopic examination is unavailable owing to anterior segment opacity (Gumpenberger and Kolm, 2006; Squarzoni et al., 2010). However, utility of the newly introduced ultrasound equipment—ultrasound biomicroscopy (UBM)— is yet to be evaluated in the Columbiformes as well as other bird species. UBM provides noninvasive imaging of the anterior ocular segment at near microscopic resolution, especially optimized for assessment of the iridocorneal angle (ICA) and ciliary cleft (CC) in veterinary species (Gibson et al., 1998; Aubin et al., 2003).

The objective of the present study is to evaluate the feasibility of UBM in avian species and to establish biometric reference ranges for normal pigeon eyes. This information would be essential for accurate interpretation of the findings of future ophthalmic examinations performed in the clinical or research setting.

Materials and Methods

1. Experimental procedures

The present study utilized ten adult pigeons (*Columba livia* var. *domestica*) that were from an avian farm raising ornamental birds. They were gently physically restrained in the sternal position. Each ophthalmic examination included several procedures. Palpebral reflexes, pupillary light reflexes, and menace responses were evaluated OU. The intraocular pressure (IOP) was measured OU using a rebound tonometer (TonoVet® ; icare, Tiolat, Helsinki, Finland) and the calibration was set to 'd'. Slit-lamp biomicroscopy (SL-D7; Topcon Corp., Tokyo, Japan) was performed OU to evaluate the anterior segments of the eyes. For fundic evaluation, indirect ophthalmoscopy was attempted but could not be conducted without pharmacological pupillary dilation.

UBM with a hand-held 50-MHz transducer (MD-320W; MEDA Co., Ltd, Tianjin, China) was performed OS after topical instillation of 0.5% proparacaine hydrochloride (Alcaine® ; Alcon, Puurs, Belgium). During UBM examinations, the eyelids were manually held open; the transducer was placed perpendicular to the dorsolateral limbus. The brightness of the light in the examination room was consistent for all pigeons, and the same UBM operator obtained all images. All of the above procedures were reviewed and approved by the Institutional Animal Care and Use Committees of the Seoul National University (SNU-160629-13).

2. Measurements and statistical analyses

Three IOP measurements with an acceptable SD (<1 mmHg) were obtained for each eye. Mean and standard deviations were calculated for all the eyes combined and for right and left eyes separately. Paired samples t-test was used to compare the IOP values obtained from the right and left eyes. A P-value of < 0.05 was considered statistically significant.

Four parameters were measured with the caliper built in the software provided with the UBM equipment: (i) CC length, the distance between the posterior end of the ciliary cleft and the corneoscleral limbus; (ii) CC width, the distance from the peripheral iris root surface to the inner surface of the cornea/sclera on a perpendicular line; (iii) CC area, the cleft area surrounded by the CC width; and (iv) ICA, the angle between the plane of the peripheral iris root and the posterior limbus. For each UBM parameter, three sets of measurements were obtained for each UBM image, with the mean values and standard deviations calculated for all the left eyes combined.

Results

Palpebral reflexes, pupillary light reflexes, and menace responses were present in all eyes. Slit lamp biomicroscopic examinations revealed richly vascularized iris in all pigeon eyes, which were otherwise unremarkable (Fig. 1). Overall mean IOP values of all eyes were 11.7 ± 1.6 mmHg with the 'd' mode and there was no statistically significant difference in the IOP between the left and right eyes ($P = 0.415$). All pigeon eyes were considered ophthalmologically normal.

Without sedation or anesthesia, all pigeons tolerated the scanning procedure well and reproducible UBM images were obtained easily from all pigeon eyes. Perpendicular orientation of the UBM probe was achieved in all images (Fig. 2), given that the bright reflections from the outer and inner corneal surfaces and anterior lens capsule were simultaneously visualized (Aubin et al., 2003; Nissirios et al., 2005).

UBM characteristics of pigeons were similar to those observed in dogs. The corneal layers were clearly differentiated in all pigeon eyes, in that the hyperechoic epithelium and Descemet's membrane were identified with the hypoechoic stroma in between. Pigeon eyes had a wide and well-developed ciliary cleft relative to their eyeball size. The measurements of CC length, CC width, CC area, and ICA are presented in Table 1.

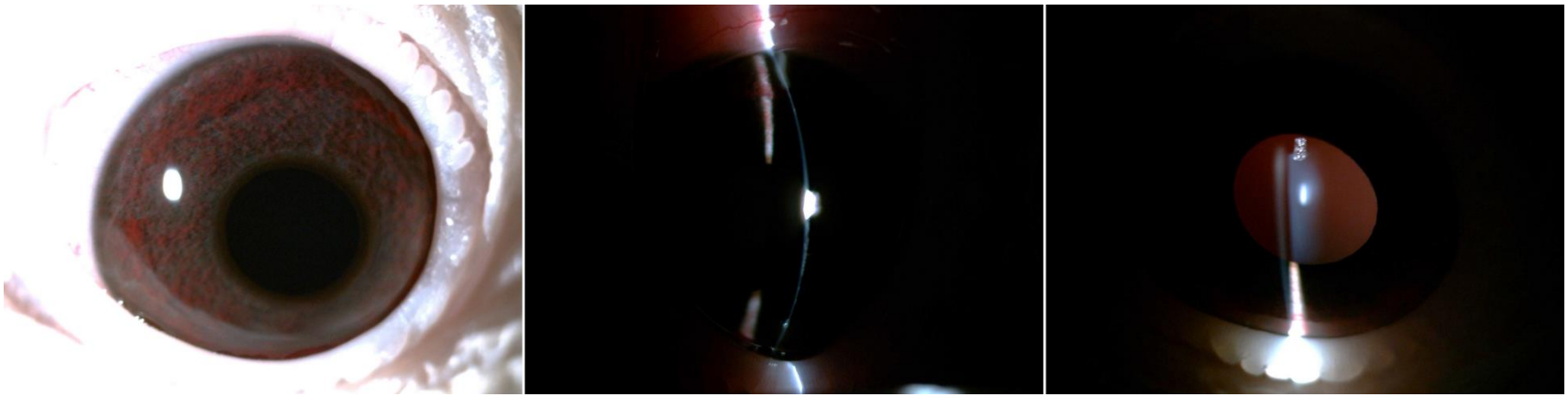

Figure 1. Slit lamp biomicroscopy in the normal pigeon eye. Note the well-developed vasculature of the iris.

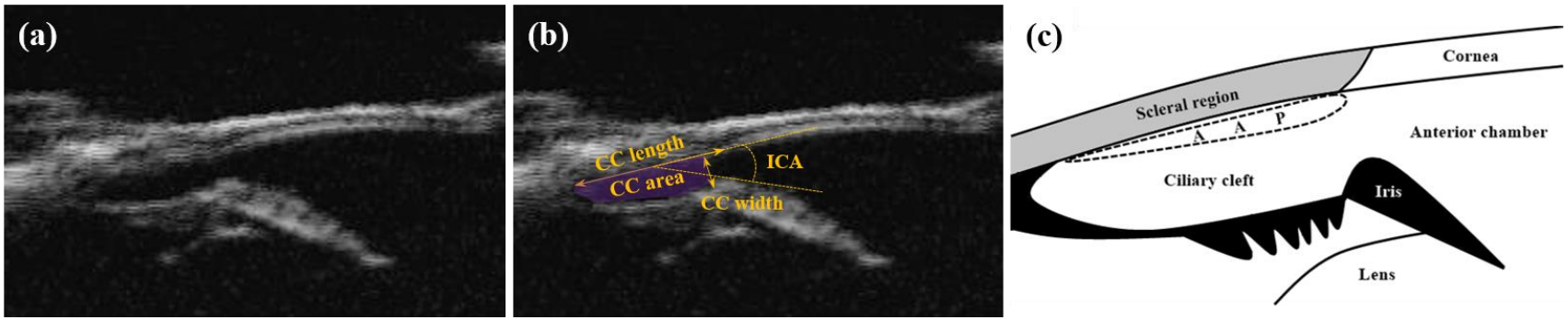

Figure 2. Ultrasound biomicroscopy (UBM) in the normal pigeon eye. (a) The hyperechoic appearance of corneal epithelium, Descemet's membrane, iris posterior epithelium, and anterior lens capsule were identified simultaneously. This indicated that the probe orientation was perpendicular to the limbus. (b) Measurements of ciliary cleft (CC)-related UBM parameters are indicated. CC length = distance between the posterior end and limbus; CC width = distance from iris root surface to the inner surface of the cornea/sclera on a perpendicular line; CC area = the cleft area surrounded by the CC width; and ICA = the angle between the plane of the peripheral iris root and the limbus. (c) Schematic diagram of the sagittal section of iridocorneal angle of pigeon eye. Angular aqueous plexus (AAP) delineated by the dotted line was not visualized by UBM.

Table 1. Biometric results of UBM parameters in pigeons (n = 6)

	Mean ± SD	Minimum	Maximum
CC length (mm)	1.61 ± 0.15	1.40	1.81
CC width (mm)	0.37 ± 0.03	0.32	0.39
CC area (mm^2)	0.40 ± 0.03	0.37	0.43
ICA (degrees)	14.81 ± 1.05	13.47	16.03

UBM = ultrasound biomicroscopy; n = number of eyes; CC = ciliary cleft; ICA = iridocorneal angle; SD = standard deviation; Minimum = lowest measurement; Maximum = highest measurement

Discussion

The vascular bed on the iris observed in the present study is a characteristic anatomical feature of the order Columbiformes (Fig. 1). These iridal vessels are arterioles branching from the annular artery and draining into the ophthalmic rete, which is located in the temporal regions of the skull in birds (Pinshow et al., 1982). The warm arterial blood flowing toward the brain cools via the countercurrent flow system in the ophthalmic rete; this allows the birds to maintain their brain temperature below their body temperature, a condition critical to protect the brain from heat stress during flight (Pinshow et al., 1982). The engorgement of iridal vessels to dissipate heat was detected during artificial heating outside the cornea (Pinshow et al., 1982). Awareness of the normal iridal vasculature and its physiology in Columbiformes would enable clinicians to avoid mistaking it for rubeosis iridis, which is an inflammatory condition.

To estimate IOP in pigeons, the Tonovet®, a rebound tonometer, was used. Since an applanation tonometer, Tonopen®, has previously been reported to limit the measurement reproducibility in birds with corneal diameter below 9 mm (Willis and Wilkie, 1999), Tonovet® was chosen for pigeon eyes, as their corneal radius was identified to be approximately 3.5 mm in a previous study (Marshall et all, 1973). Tonovet® has 3 different measurement settings: ‘d’ for dogs and cats, ‘h’ for horses, and ‘p’ for other species. As there is no known calibration table for pigeons, the ‘d’ mode was selected arbitrarily in the present study and the IOP in the ‘d’ mode was determined to be 11.7 ± 1.6 mmHg in pigeons. The differences in

corneal curvature and corneal thickness could contribute to variations in IOP values between and/or within species (Park et al., 2011; Jeong et al., 2007). Further study is warranted to evaluate the accuracy of Tonovet®, in comparison with manometrically set IOP, in pigeon eyes and to establish a calibration table for pigeons.

B-mode ultrasonography has proved to be a useful diagnostic tool in avian ophthalmology (Gumpenberger and Kolm, 2006; Amber et al., 2012; Lehmkuhl et al., 2010). An ultrasonographic study of pigeon eyes revealed that pigeons have globular shaped globes (Gumpenberger and Kolm, 2006). Although the avian eye fits tightly in the orbit, the dorsotemporal area is unprotected by bone (Bayon et al., 2007), enabling appropriate placement of the UBM probe in this area and acquisition of reproducible angle images. The avian sclera is supported by the 10 to 18 bony ossicles forming a sclerotic ring (Bayon et al., 2007; Hall, 2008). In an ultrasonographic study of pigeons, the hyperechoic sclerotic ring hindered measurement of the globe diameter by creating distal shadowing (Gumpenberger and Kolm, 2006). However, in the present study, the angle anatomy was well observed through the corneoscleral limbus using UBM, which revealed that the iridocorneal angle of pigeons consisted of anteriorly separated inner and outer leaves of the ciliary body forming the ciliary cleft as is observed in dogs, cats, and horses (Fig. 2a) (Gibson et al., 1998; Aubin et al., 2003). Although the values of CC parameters of pigeons were mostly lower than those of dogs owing to the small size of pigeon's globe, the mean CC width of pigeons was exceptionally similar to that of dogs, measured as being 0.37 ± 0.06 mm in a previous canine study (Kwak

et al., 2016). This would be a supporting piece of evidence for the concept that pigeons possess a relatively wide and well-developed ciliary cleft.

Compared with other vertebrate eyes, the size of the angular aqueous plexus in birds is known to be remarkably large, 0.3–1 mm in length and 0.03–0.09 mm in width in a sagittal section (Fig. 2c) (Tripathi and Tripathi, 1973). However, UBM could not delineate the angular aqueous plexus in the present study. Although the 50 MHz UBM allows discrimination of structures as small as 50 μm (Gibson et al., 1998), the resolution of the equipment would seem to be insufficient to visualize the angular aqueous plexus of pigeons.

Among domestic animals, horses are also considered to have a well-developed angular aqueous plexus (Gum and Mackay, 2013). In an ultrastructural study of equine iridocorneal angle, the width of the angular aqueous plexus was measured to be 0.02–0.05 mm, while the distance between the limbus and the iridal base was around 1.4 mm (Geest et al., 1990). The mean CC width, which is an UBM approximation of the distance between the limbus and the iridal base, was 0.36 mm in the current study, and the maximal width of the angular aqueous plexus of pigeons in a previous histological study was 0.09 mm (Tripathi and Tripathi, 1973), that accounts for a quarter of the UBM-measured CC width in pigeons. And since the resolution of UBM equipment was insufficient to clearly differentiate the angular aqueous plexus from the ciliary cleft, there could be an inherent tendency to overestimate the UBM measurements of CC-related parameters in pigeons.

It is assumed that the size of the ciliary cleft and angular aqueous plexus in

avian species could be related to their high aqueous outflow facility (Tripathi and Tripathi, 1973). Avians accommodate by changing the corneal curvature and by thickening the lens causing it to bulge into the anterior chamber (Ofri, 2013). The cumulative results of anatomical changes during accommodation would reduce the volume of the anterior chamber and this could be compensated by their well-developed ciliary cleft and angular aqueous plexus (Tripathi and Tripathi, 1973).

As the small sample size could limit the wide application of the reference ranges established in this study, further study with larger population is warranted to increase the precision of the estimates. Although the UBM was performed unilaterally in a conscious state in the present study to reduce handling stress of the pigeons, the use of sedatives or anesthetic agents might be helpful to obtain clear UBM images for longer scanning procedure in birds for their safety. Prior to the use of sedatives or anesthetic agents in the UBM scanning procedure, however, it is necessary to evaluate their influences on the ocular anterior segment anatomy.

Conclusions

UBM could be useful for the qualitative and quantitative assessment of the anterior ocular segment of pigeons without the need for sedation or general anesthesia. However, it is noteworthy that the presence of the large angular aqueous plexus above the ciliary cleft might overestimate the size of the ciliary cleft.

CHAPTER II

Ultrasound biomicroscopic study of the effects of topical latanoprost on the anterior segment and ciliary body thickness in dogs

Abstract

The goal of this study was to evaluate the effects of topical latanoprost on the anterior segment and ciliary body using ultrasound biomicroscopy (UBM) in dogs. This study included six eyes of 6 clinically normal beagles. UBM scans were performed on 6 sedated dogs before and 2 hours after topical latanoprost instillation. From the next day on, latanoprost was topically applied twice daily for 7 days. After 1 week of instillation, the UBM scans were repeated. The ciliary body thickness (CBT) as well as the anterior segment parameters, including the iridocorneal angle (ICA), the width of the ciliary cleft (CC) entry, the length of the CC, and the width of the mid-CC were measured. The topical latanoprost decreased the ICA and CC entry width and increased the mid-CC width without any significant alterations in the CC length. There were time-dependent alterations in the CBT: a reduction in the CBT after 2 hours of instillation and rebound thickening after 1 week of instillation. The topical latanoprost widened the ciliary cleft despite the narrowing of the ICA and CC entry. Time-dependent alterations in the CBT were demonstrated by the UBM and might be a reflection of the mechanism of the uveoscleral outflow enhancement induced by the topical latanoprost.

Introduction

Currently, topical latanoprost is an effective hypotensive drug commonly used for the treatment of primary glaucoma in dogs (Miller, 2013). Although the ocular hypotensive mechanism of topical latanoprost has not been fully elucidated in dogs, there is some evidence suggesting that latanoprost enhances the uveoscleral outflow in dogs as in humans (Tsai et al., 2012; Plummer et al., 2013). In a recent report, an increase in the episcleral venous pressure (EVP) was observed after the dosing of 0.005% latanoprost in dogs (Tsai et al., 2012). Considering the pressure-independent nature of the uveoscleral pathway, an EVP increase in conjunction with the intraocular pressure (IOP) lowering effect of latanoprost may reflect reduced aqueous production and/or increased uveoscleral outflow (Tsai et al., 2012). While one preliminary study suggested that 0.005% latanoprost could reduce aqueous humor production (Ward, 2005), no conclusive evidence indicating increased uveoscleral outflow by topical latanoprost has ever been reported in the canines. Since the uveoscleral pathway is through the ciliary muscle interstitium (Tsai et al., 2012; Plummer et al., 2013) and is thought to be related to the contraction and relaxation of the ciliary muscle (Tsai et al., 2012; Mishima et al., 1996; Alm and Nilsson, 2009), monitoring structural modifications of the ciliary musculature induced by latanoprost might be the most effective way to verify the uveoscleral outflow involvement in the effectiveness of topical latanoprost.

It has been suggested that there might be time-dependent variation in the mechanism of the ocular hypotensive effect of latanoprost (Plummer et al., 2013;

Alm and Nilsson, 2009). The early effect of prostaglandins on the uveoscleral outflow is considered to be mediated by a relaxation of the ciliary muscle, and the long-term effect is likely to be mediated by matrix metalloproteinase (MMP)-mediated dissolution of the extracellular matrix (ECM) around the ciliary muscle bundles (Alm and Nilsson, 2009). Both the ciliary muscle relaxation and the remodeling of the interstitial spaces of the ciliary musculature could contribute to the widening of the spaces around the muscle bundles and resultant increased uveoscleral outflow.

The introduction of the high-resolution ultrasound biomicroscope (UBM) has allowed noninvasive in vivo imaging of the anterior segment of the eye at about the same power as a histological examination (Bentley et al., 2005). Although another newly introduced equipment, anterior segment optical coherence tomography (AS-OCT) also provides higher imaging resolution than UBM, it cannot clearly image beyond the pigmented epithelium of the iris due to the light absorption by this layer (Dorairaj et al., 2007). In other words, the UBM is superior to AS-OCT for the imaging of the ciliary musculature posterior to the iris.

In order to evaluate the time-based effects of topical latanoprost on the uveoscleral pathway, the anterior segment structures and the ciliary body (consisting of the ciliary muscle bundles and their interstitium) were imaged at specific time points during the study period and their dimensions were measured using UBM.

Materials and Methods

1. Scanning procedures

Six eyes from 6 clinically normal beagles were imaged using UBM with a handheld 50-MHz transducer (MD-320W, MEDA Co., Ltd, China). All dogs used in the study underwent ophthalmic examinations including slit lamp biomicroscopy, rebound tonometry, and indirect ophthalmoscopy and were ophthalmologically normal. The study design was as follows. All dogs were sedated with 0.1 mg/kg acepromazine (Sedaject® , Samwoo Medical, Chungnam, South Korea) IV 30 minutes before the procedure. They were examined with manual restraint in sternal recumbency after the topical instillation of 0.5% proparacaine hydrochloride (Alcaine® , Alcon, Puurs, Belgium). The eyelids were manually held open; the transducer was placed perpendicular to the limbus at the 12 o'clock position, and baseline images were obtained from all animals. Subsequently, one drop of 0.005% latanoprost (Xalatan® ; Pfizer, Inc., New York, NY) was applied topically, and the UBM scan was repeated 2 hours later. To maintain the degree of sedation constant for all scanning procedures, acepromazine IV was repeated when needed. From the next day on, the latanoprost was topically applied twice daily for 7 days. After 2 hours of the last dosing of latanoprost, the UBM scans were performed again under the same level of sedation at the same time of day. The brightness of the light in the exam room was consistent for all scanning procedures, and the same UBM

operator obtained all images throughout the study. Several images were captured during the scanning procedures and the best three among them were used for measurements. Only images showing all iridocorneal angle structures (corneoscleral limbus, iris root, and ciliary cleft), specifically including the posterior end of ciliary cleft, were selected for evaluation. All of the above procedures were reviewed and approved by the Institutional Animal Care and Use Committees of the Seoul National University (SNU-140731-1).

2. Anterior segment parameters

The following parameters, as defined by Dulaurent et al (Dulaurent et al., 2012), were assessed for each obtained image (Fig. 3): 1) iridocorneal angle (ICA) formed by the plane of the peripheral iris root and the posterior limbus, 2) the width of the ciliary cleft (CC) entry, the distance between the limbus and the peripheral iris root, 3) the CC length, the distance between the most anterior visible portion of the uveal trabecular meshwork and the posterior end of the CC, and 4) the mid-CC width, the width of the ciliary cleft at the center of the CC.

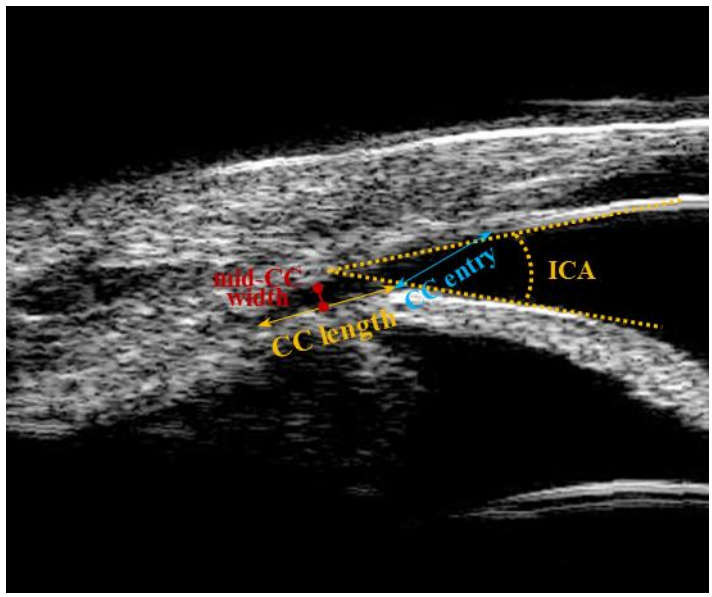

Figure 3. Measurements of anterior segment parameters. ICA, iridocorneal angle formed by the plane of the peripheral iris root and the posterior limbus; CC entry, the distance between the limbus and the peripheral iris root; CC length, the distance between the most anterior visible portion of the uveal trabecular meshwork and the posterior end of ciliary cleft; mid-CC width, the width of ciliary cleft at the center of the ciliary cleft.

3. Ciliary body thickness parameter

In addition to the parameters for the anterior segment evaluation, we measured the UBM thickness of the ciliary body as well. As the attached, outer portion of the ciliary body is negligible compared with the inner portion, 10 the ciliary body thickness (CBT) was defined as the distance between the posterior end of the CC and the edge of the ciliary pigmented epithelium, which appeared hyperechoic on the UBM images (Fig. 4).

4. Measurements and statistical analyses

All measurements were made with the built-in caliper in the software included within the UBM equipment. For all parameters, measurements were performed in triplicate for each image, with the mean values and standard deviations calculated. The repeated measures ANOVA was used to test the difference in each parameter before and after the topical instillation.

In addition, we investigated intra-observer reproducibility because the CBT parameter was first introduced in this study. For the intra-image comparison, the intraclass correlation coefficient (ICC) was calculated on one image only, randomly chosen from the three selected images of each eye. Statistical analyses were performed using the SPSS software. ICC values higher than 0.900 were considered to indicate a good agreement, and a P value of less than 0.05 was considered to be statistically significant.

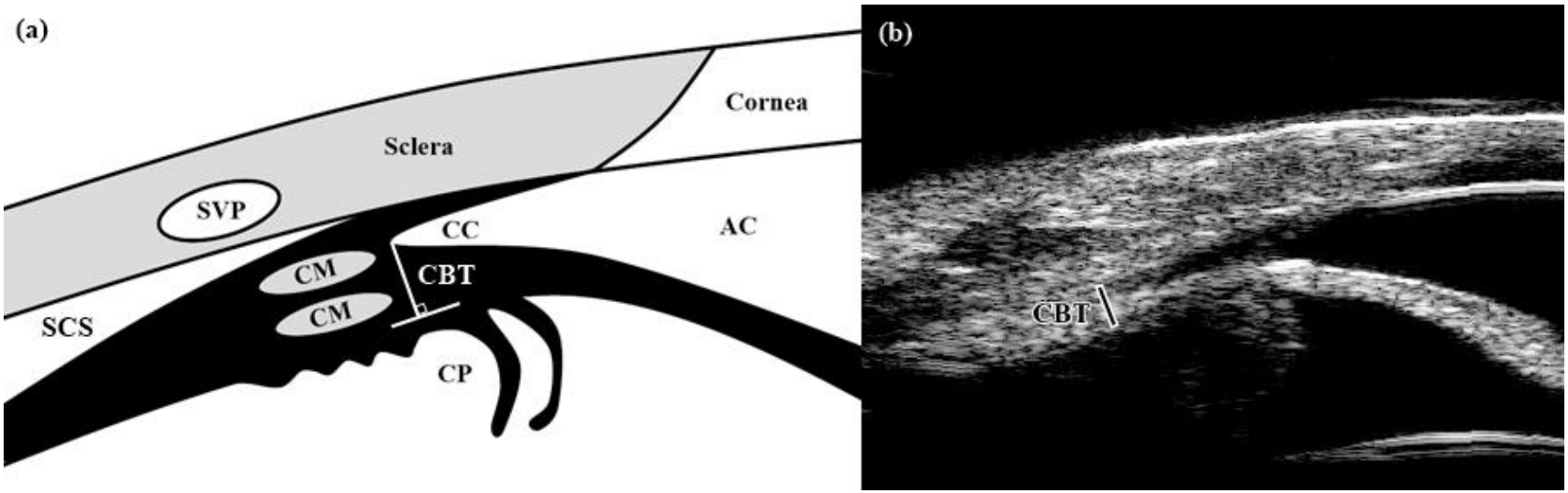

Figure 4. Basic concepts of the measurement of ciliary body thickness (CBT). Determination of the CBT parameter on the ultrasound biomicroscope (UBM) is based upon the similarity between light microscopy and ultrasound biomicroscopy. (a) Schematic diagram of the sagittal section of the iridocorneal angle tissues in dogs. AC, anterior chamber; CC, ciliary cleft; CM, ciliary muscle bundle; CP, ciliary process; SCS, supraciliary space; SVP, scleral venous plexus. (b) UBM image in the sagittal plane. CBT could be measured from the posterior end of CC to the edge of the ciliary pigmented epithelium.

Results

1. Intra-observer reproducibility of measurements

All of the ICC values were higher than 0.900 throughout 3 scanning procedures (Table 2). Because the measurement of the ICA involved making two reference lines unlike the other parameters, the reproducibility of the ICA was the lowest among them. The newly introduced parameter, CBT, showed good reproducibility, similar to those of other UBM parameters. However, ICCs of the mid-CC width and the CBT were relatively smaller than the others, indicating that the hypoechoic appearance of the ciliary cleft caused its border to appear ill-demarcated, leading to the lesser intra-observer reproducibility.

Table 2. Intra-observer reproducibility of UBM parameters

UBM parameters	Baseline		After 2 hours		After 1 week	
	ICC	95% CI	ICC	95% CI	ICC	95% CI
ICA	0.940	0.745-0.973	0.977	0.903-0.997	0.977	0.901-0.996
CC entry	0.998	0.992-1.000	0.998	0.990-1.000	0.996	0.982-0.999
CC length	0.992	0.965-0.999	0.995	0.979-0.999	0.995	0.978-0.999
CC mid width	0.989	0.955-0.998	0.964	0.850-0.995	0.991	0.963-0.999
CBT	0.992	0.965-0.999	0.974	0.889-0.996	0.982	0.923-0.997

UBM, ultrasound biomicroscopy; ICA, iridocorneal angle; CC, ciliary cleft; CBT, ciliary body thickness; ICC, intraclass correlation coefficient; CI, confidence interval

2. Anterior segment parameters

Among the anterior segment parameters, the ICA, the CC entry width, and the mid-CC width showed statistically significant changes (Fig. 5). As the peripheral iris approached the limbus after the latanoprost instillation, the latanoprost significantly narrowed the ICA and the CC entry, regardless of the duration of instillation. The mean ICA significantly decreased from 30.93 ± 4.08 degrees to 20.35 ± 2.06 degrees after 2 hours of instillation (P = 0.005) and to 21.39 ± 2.54 degrees after 1 week of instillation (P = 0.004). The mean CC entry width decreased from 1.33 ± 0.24 mm to 1.10 ± 0.30 mm after 2 hours of instillation (P = 0.023) and to 1.11 ± 0.24 mm after 1 week of instillation (P = 0.010). In contrast, the mean mid-CC width increased from 0.34 ± 0.07 mm to 0.40 ± 0.05 mm after 2 hours of instillation (P = 0.007), and to 0.42 ± 0.06 mm after 1 week of instillation (P = 0.010).

The other parameter, the CC length was not significantly changed throughout the study period. The mean CC length was 1.97 ± 0.15 mm before the instillation, 1.90 ± 0.18 mm after 2 hours of instillation, and 1.90 ± 0.14 mm after 1 week of instillation.

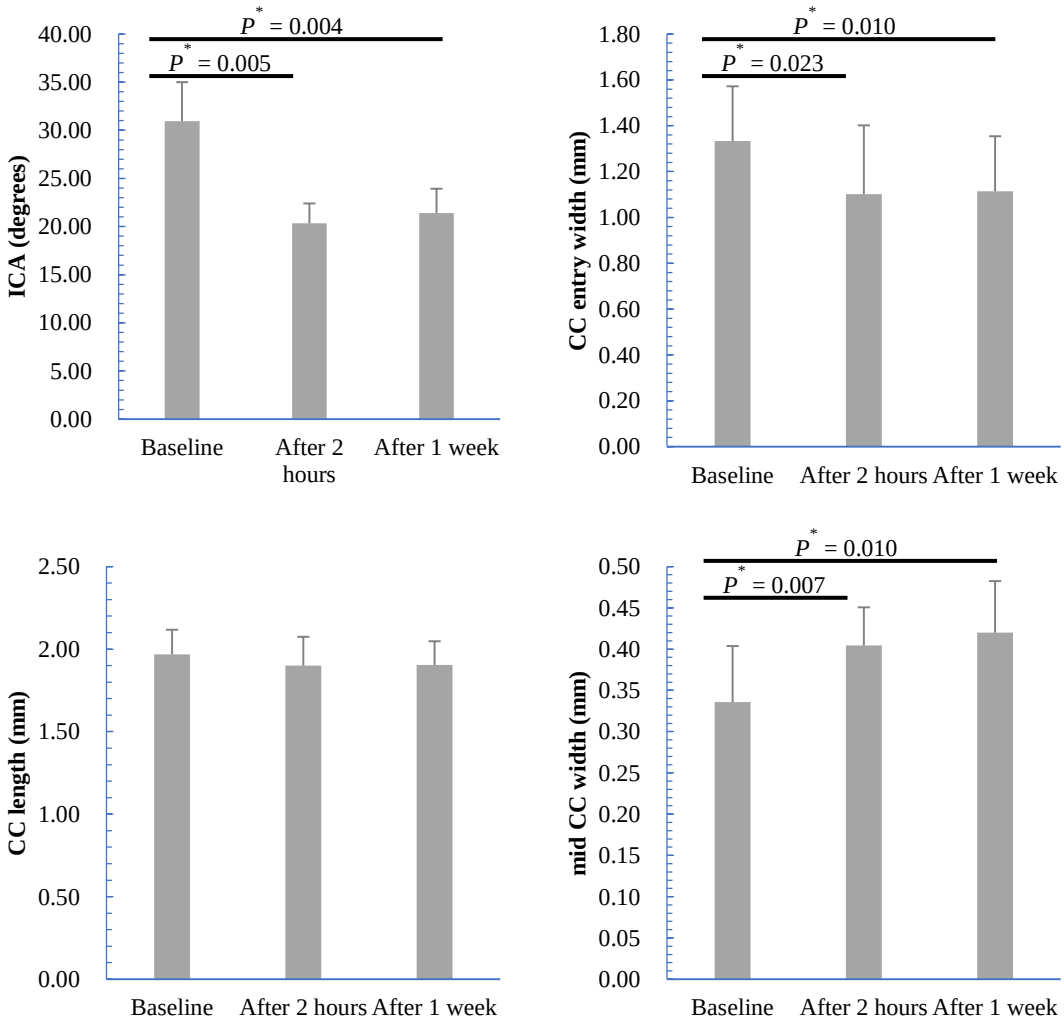

Figure 5. Effects of latanoprost on anterior segment parameters. The topical latanoprost significantly decreased the iridocorneal angle (ICA) and the width of ciliary cleft (CC) entry and increased the mid-CC width without any significant changes in the CC length. * $P < 0.05$

3. Ciliary body thickness

The mean CBT significantly decreased about 13%, from 0.52 ± 0.07 mm to 0.45 ± 0.06 mm after 2 hours of instillation (P = 0.004). However, after 1 week of twice daily instillation, the mean CBT rose back to the baseline value of 0.51 ± 0.08 mm (P = 0.012) (Figs 6 and 7).

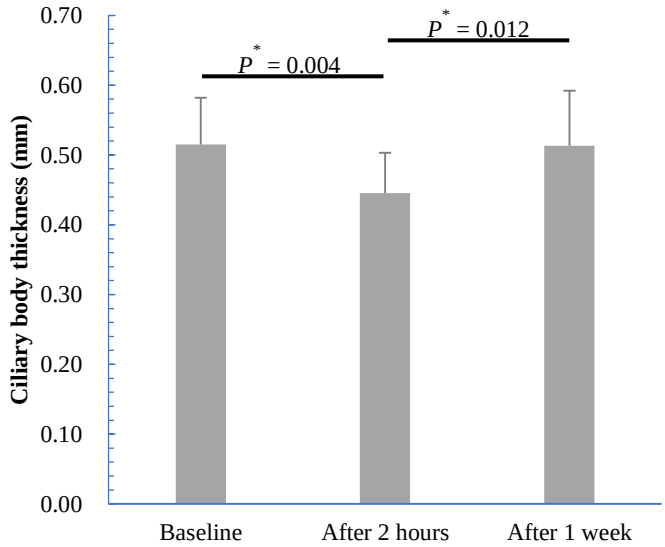

Figure 6. Effects of latanoprost on ciliary body thickness (CBT). The mean CBT was significantly reduced after 2 hours of instillation and restored its baseline thickness after 1 week of instillation. * $P < 0.05$.

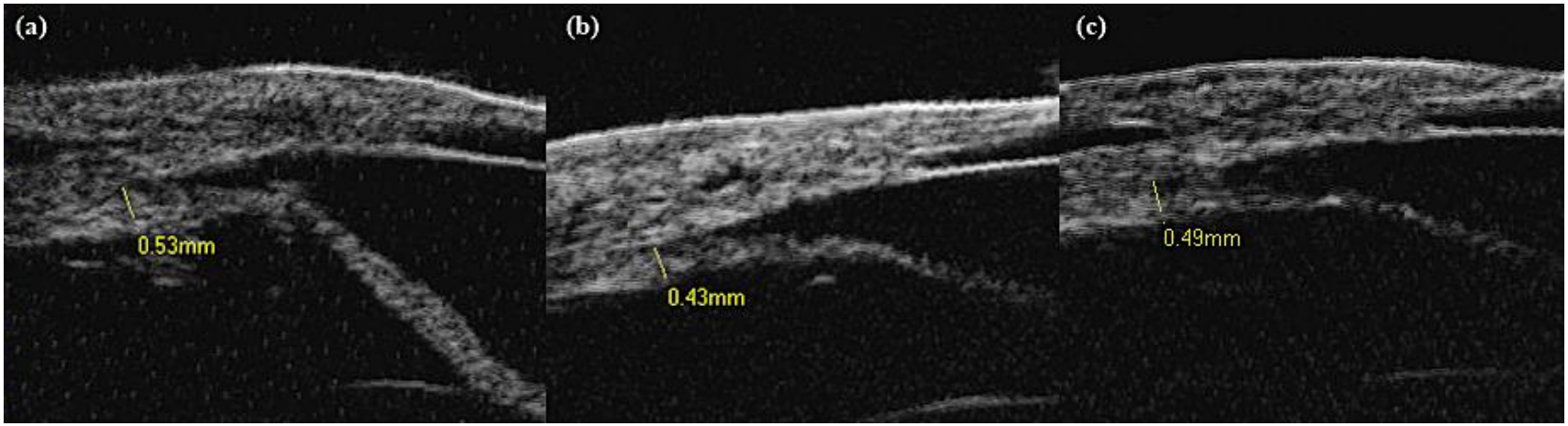

Figure 7. Representative images of the ciliary body thickness (CBT) changes in the same eye. (a) Before latanoprost instillation. (b) After 2 hours of latanoprost instillation. (c) After 1 week of latanoprost instillation.

Discussion

The lack of the scleral spur which is a commonly used reference point in human UBM studies, and the presence of the ciliary cleft originating from the bi-leaflet configuration of the ciliary body in dogs (Samuelson and Streit, 2012; Samuelson, 2013; Carreras et al., 1997) led us to use a different methodology from human for the anterior segment biometry with the UBM. An earlier study has already suggested that considering the ciliary cleft and iridocorneal angle as different structures would be more helpful to understand the aqueous outflow dynamics in dogs (Dulaurent et al., 2012). Since the ciliary cleft refers to the region located between the anterior leaves of the ciliary body (Samuelson, 1996), they suggested several ciliary cleft parameters to distinguish the ciliary cleft from the iridocorneal angle (Dulaurent et al., 2012).

In the present study using those parameters (Dulaurent et al., 2012), topical latanoprost induced a significant reduction in the ICA and the width of the CC entry. This may be due to the anterior displacement of the peripheral iris root and the resultant closer approximation between the limbus and the peripheral iris root, which was consistent with previous findings by AS-OCT (Tsai et al., 2013). In spite of the narrowing of the CC entry, the mid-CC width was increased after the latanoprost instillation. Additionally, due to the fact that the canine trabecular meshwork is located at the most posterior portion within the CC recess (Dulaurent et al., 2012; Samuelson, 1996; Bedford, 1986), the aqueous outflow may not be compromised functionally with the narrowing of the ICA following latanoprost

instillation.

While there are several reports about the in vivo imaging of the ciliary muscle response to various pharmacological agents in humans (Mishima et al., 1996; Marchini et al., 2003), similar studies are rare in veterinary ophthalmology. Because the human CBT parameter is usually measured at a specific distance away from the scleral spur which does not exist in dogs (Dorairaj et al., 2007), the canine-specific CBT parameter should be defined anew. Since the obtained images were in the sagittal plane (Rose et al., 2008), we referred to the sagittal sections of the ICA and ciliary body in other canine histopathological studies to find a clue to the measurement of ciliary muscles and their interstitial spaces (Samuelson and Streit, 2012; Samuelson, 1996). Smooth muscle bundles in the anterior outer portion of the ciliary body musculature are negligible when compared to the anterior inner portion (Samuelson and Streit, 2012), and the attached, outer portion holds the body of the ciliary muscle, which is exclusively longitudinal and situated posteriorly (Fig. 4a) (Carreras et al., 1997; Bedford, 1986). Therefore, the ciliary body thickness could be defined as a distance from the posterior end of the CC which is right beneath the thin, attached portion of the ciliary body to the hyperechoic edge of the ciliary pigmented epithelium (Fig. 4b).

Our findings about ciliary body thickness showed that the CBT decreased by about 13% after 2 hours of topical latanoprost. This decreasing tendency was in agreement with a human study, where the CBT decreased approximately 3.8% after 4 hours of the application of the $PGF_2\alpha$ analog ($P < 0.01$) (Mishima et al., 1996).

The discrepancy between the degrees of reduction in the CBT may be linked to the different levels of prostaglandin E_2 (PGE_2) between dogs and humans. The latanoprost-induced PGE_2 level in isolated ciliary muscles was reported to be much higher in dogs than in humans (Yousufzai et al., 1996). Since the $PGF_2\alpha$-induced PGE_2 release was dose-dependent (Yousufzai et al., 1996), and the PGE_2 exhibited the strongest ciliary muscle relaxation effect of all prostaglandins (Goh and Kishino, 1994), the higher PGE_2 level might contribute to the lesser CBT values in dogs than in humans.

The early reduction in the CBT was followed by the rebound thickening of the ciliary body after 1 week of the twice daily instillation of topical latanoprost. The CBT was restored to almost the same as its baseline value. In humans, however, the CBT after the 1 week instillation of latanoprost exceeded the baseline values, showing a 25% to 30% increase in the CBT (Marchini et al., 2003). As the last scan was performed 2 hours post-dosing in the present study, the effect of ciliary muscle relaxation could counteract the CBT increase at the time of the last scanning procedure. Considering the degrees of the CBT reduction after the first dosing, however, the extent of ciliary body expansion after 1 week administration was below that of humans (Marchini et al., 2003). The different increasing capacity between two species could be ascribed to the development degree of the ciliary body musculature. Dogs have less-developed ciliary body musculature than primates (Alm and Nilsson, 2009; Samuelson, 2013), indicating the possibility of an inherent limitation to tissue expansion. Generally, PG analogues are instilled once daily in humans, in accordance with the manufacturer's recommendations

(Plummer et al., 2013). Although the twice daily instillation of latanoprost is considered to be less effective than a once daily instillation in human glaucoma patients (Plummer et al., 2013; Linden and Alm, 1998), the twice daily instillation produced the greatest decline in the IOP with the least diurnal IOP fluctuation in a canine study (Gelatt and Mackay, 2001). This reduction of efficacy and the resultant frequent dosing schedule in dogs might be related to the limited capacity of the ciliary body expansion observed in the present study.

In conclusion, the findings in this study, the reduction of CBT after a single instillation and the increasing tendency of CBT after a week administration, represented the early and the long-term effect of latanoprost on the uveoscleral pathway, respectively. These observations indirectly support the well-established hypothesis that the early effect of latanoprost might be connected to widening of intermuscular spaces as a result of ciliary muscle relaxation and the long-term effect to enlargement of empty spaces between the ciliary muscle bundles following ECM degradation. Although all these structural changes contribute to reducing the hydraulic resistance through the uveoscleral outflow, the relationship with ocular hypotensive effect has yet to be identified as their influence on IOP was not investigated in the current study. In addition, the rapid effect of latanoprost to alleviate an attack of primary angle-closure glaucoma in dogs was suggested to be associated with miosis by Miller et al., which is irrelevant to the aforementioned findings obtained herein with the use of normotensive Beagle dogs. Clinical significance of these findings and their association with functional influence on IOP remain to be determined.

There are still several limitations to this study. In regard to the use of acepromazine as a sedative, the possibility of ocular effects from acepromazine could not be entirely excluded. Although the ocular effect of systemically administered acepromazine has not yet been reported to date, there is a study demonstrating that pupil size and IOP were unaffected by topically administered acepromazine in monkeys (Hayreh et al., 1991). Furthermore, miosis and angle narrowing were not caused by an anesthetic regimen including acepromazine in canine studies (Tsai et al., 2013; Almazan et al., 2013). Therefore, acepromazine might not be related to the observed changes in the present study.

Besides the small sample size, it is possible that 1 week, the duration of the latanoprost instillation in this study, is not long enough to expect the occurrence of MMP-mediated changes in the ciliary body in dogs. In addition, the time-dependent CBT alterations found in this study might be elicited by miosis, rather than the direct action of latanoprost on the ciliary body. Under the influence of parasympathomimetic miotics, it was found that the iris pulled the detached ciliary body centrally toward the posterior chamber irrespective of the ciliary body expansion (Carreras et al., 1997). Therefore, further studies on the ciliary muscle status with relation to miosis need to be done to clarify whether CBT alterations following latanoprost instillation are PG-related effects or not.

Conclusions

Topical latanoprost widened the ciliary cleft despite the narrowing of the ICA and CC entry. Time-dependent alterations in the CBT were demonstrated by the UBM and might be a reflection of the mechanism of the uveoscleral outflow enhancement induced by the topical latanoprost.

CHAPTER III

Effects of prostaglandin-mediated and cholinergic-mediated miosis on morphology of the ciliary cleft region in dogs

Abstract

This study was to compare morphology of the ciliary cleft (CC) region in dogs after topical administration of latanoprost, pilocarpine, and a combination of latanoprost and pilocarpine. Six Beagles were used. A prospective crossover study with washout period between 4 phases was performed. Latanoprost (phase L), pilocarpine (phase P), pilocarpine followed by latanoprost (phase PL), and latanoprost followed by pilocarpine (phase LP) were administered to the right eye. Artificial tears were administered to the left eye (control eye). For each phase, pupil diameter and intraocular pressure (IOP) were measured and ultrasound biomicroscopy was performed 2 hours after topical treatment. Angle opening distance (AOD), ciliary cleft width (CCW), ciliary cleft length (CCL), and ciliary cleft area (CCA) were evaluated. All treated eyes had marked miosis without significant difference in pupil diameter among phases. Significant IOP reductions were detected for all phases, except phase P. AOD and CCA were significantly increased in all phases for treated eyes, compared with results for control eyes. CCW was significantly increased in phases P, PL, and LP, and CCL was significantly increased in phases PL and LP. Comparison of treated eyes among phases revealed that CCW differed significantly between phases L and P and between phases L and PL. Prostaglandin-mediated and cholinergic-mediated miosis caused variations in CC configurations. When latanoprost and pilocarpine were used in combination, the first drug administered determined the cleft morphology, which was not fully reversed by the second drug. CC morphology did not fully explain IOP reductions.

Introduction

Latanoprost and pilocarpine are effective ocular hypotensive drugs for dogs and humans. Latanoprost, a prostaglandin analogue, reduces intraocular pressure (IOP) in humans and other primates by initial relaxation of the ciliary muscle and remodeling of the extracellular matrix between the muscle bundles over time, thus facilitating uveoscleral outflow (Toris et al., 2008; Toris et al., 2001). Pilocarpine, a cholinergic agonist, contracts the ciliary muscle bundles to pull on the scleral spur, thus opening fluid channels in the trabecular meshwork and increasing trabecular outflow (Grierson et al., 1978). Despite the antagonistic mechanism of action on the ciliary muscle status, additive effects of latanoprost with pilocarpine have been suggested in human clinical studies (Toris et al., 2001; Toris et al., 2002; Kent et al., 1999). Slightly greater reductions in IOP, although not significantly different, was also detected after a combination of latanoprost and pilocarpine was administered to dogs (Sarchahi et al., 2012).

In contrast to effects in humans and other primates, prostaglandin-mediated miosis is commonly described in dogs, cats, and horses (Plummer et al., 2013). Among the prostaglandins, prostaglandin F_2 alpha is the most potent for contracting the iris sphincter muscle of dogs (Yoshitomi and Ito, 1988). Miosis has been regarded as an important side effect (Sarchahi et al., 2012; Plummer et al., 2013); however, it has been suggested (Miller et al., 2003) that latanoprost-induced miosis could play an important role in rapid reduction of IOP by resolving the pupillary block (contact of the pupillary margins with the lens) in dogs with

primary closed-angle glaucoma. In addition, cholinergic miotics have been used for the long-term treatment of dogs with open-angle glaucoma (Plummer et al., 2013). During cholinergic-mediated miosis, the iris reportedly pulls the inner leaflet of the ciliary body rostrally and centrally, which results in widening of the ciliary cleft (CC) (Carreras et al., 1997). However, few studies have been conducted to investigate differences between prostaglandin-mediated and cholinergic-mediated miosis.

The purpose of the study reported here was to investigate differences in morphology of the CC region between prostaglandin-mediated and cholinergic-mediated miosis in dogs. We hypothesized that there would be structural differences associated with their pharmacological action on ciliary muscle contractility. Ultrasound biomicroscopy (UBM) was used to describe the dynamic morphology of the anterior ocular segment in vivo. Furthermore, we sought to identify possible additive effects of pilocarpine and latanoprost in dogs and the relationship between those effects and structural alterations.

Materials and Methods

1. Animals

Six laboratory Beagles were used in the study. All dog eyes were ophthalmologically normal, as determined on the basis of results of a complete ophthalmic examination, including slit-lamp biomicroscopy (SL-D7, Topcon Corp, Tokyo, Japan), indirect ophthalmoscopy (Vantage plus, Keeler, Windsor, England), and rebound tonometry (TonoVet, iCare, Helsinki, Finland). Experimental procedures were reviewed and approved by the Institutional Animal Care and Use Committee of the Seoul National University (SNU-160613-19).

2. Experimental design

All dogs were acclimatized to experimental procedures for 3 days before the start of the experiments. At the same time on each of those 3 days, rebound tonometry was performed 3 times; a topical anesthetic was then applied to each eye and the iridocorneal angle was evaluated by use of UBM.

A prospective crossover study consisting of 4 phases was conducted; washout period between phases was ≥ 5 days. For each phase, IOP was measured in both eyes of all dogs by use of a rebound tonometer. Then, all dogs received 1 drop of 0.005% latanoprost (Xalatan, Pfizer Inc, New York,

NY) (phase L), 1 drop of 2% pilocarpine (Isopto carpine, Alcon Korea, Seoul, Republic of Korea) (phase P), 1 drop of pilocarpine followed 5 minutes later by 1 drop of latanoprost (phase PL), and 1 drop of latanoprost followed 5 minutes later by 1 drop of pilocarpine (phase LP) in the right eye (drug-treated eye). One drop of artificial tears (Lacure, Samil Pharm Co Ltd, Seoul, Republic of Korea) (placebo treatment) was administered to the left eye (control eye) of each dog for phases L and P, and 2 drops of artificial tears were administered 5 minutes apart to the control eye in phases PL and LP.

Measurement of pupil diameter and IOP as well as UBM were performed on both eyes 2 hours after topical treatment. Three valid IOP measurements were obtained with the tonometer, and the mean values was calculated for each eye in each phase. A Jameson caliper was used to measure the maximal horizontal pupil diameter. Anesthetic (0.5% proparacaine hydrochloride) was applied topically. The eyelids were manually held open, and UBM was performed with a handheld 50-MHz transducer placed perpendicular to the limbus at the 12 o'clock position. All experimental procedures were performed in the same quiet examination room by the same investigator (SP). Dogs were not sedated for experimental procedures. Although the light intensity was not measured, the dimmer switch in the examination room was set at the same intensity throughout the experiments.

3. Ultrasound biomicroscopic measurements

Only images with all the anatomic landmarks (corneoscleral limbus, iris root, CC, and anterior lens capsule) were selected for analyses (Figure 8). Considering the unmasked and nonrandomized nature of the study, each image was analyzed 5 separate times, with an interval of at least 1 week between analyses, to minimize operator bias and reduce subjectivity.

Several variables were measured with the built-in caliper in the UBM software (Figure 9). These included the AOD (distance on a perpendicular line from the surface of the peripheral iris root to the inner surface of the cornea or sclera), CCW (distance from the internal border of the outer leaflet of the ciliary body to the internal border of the inner leaflet of the ciliary body at the caudal end of the CC), CCL (distance between the caudal end of the CC and the surface of the peripheral iris root), and CCA (cleft area surrounded by the internal border of the CC and AOD).

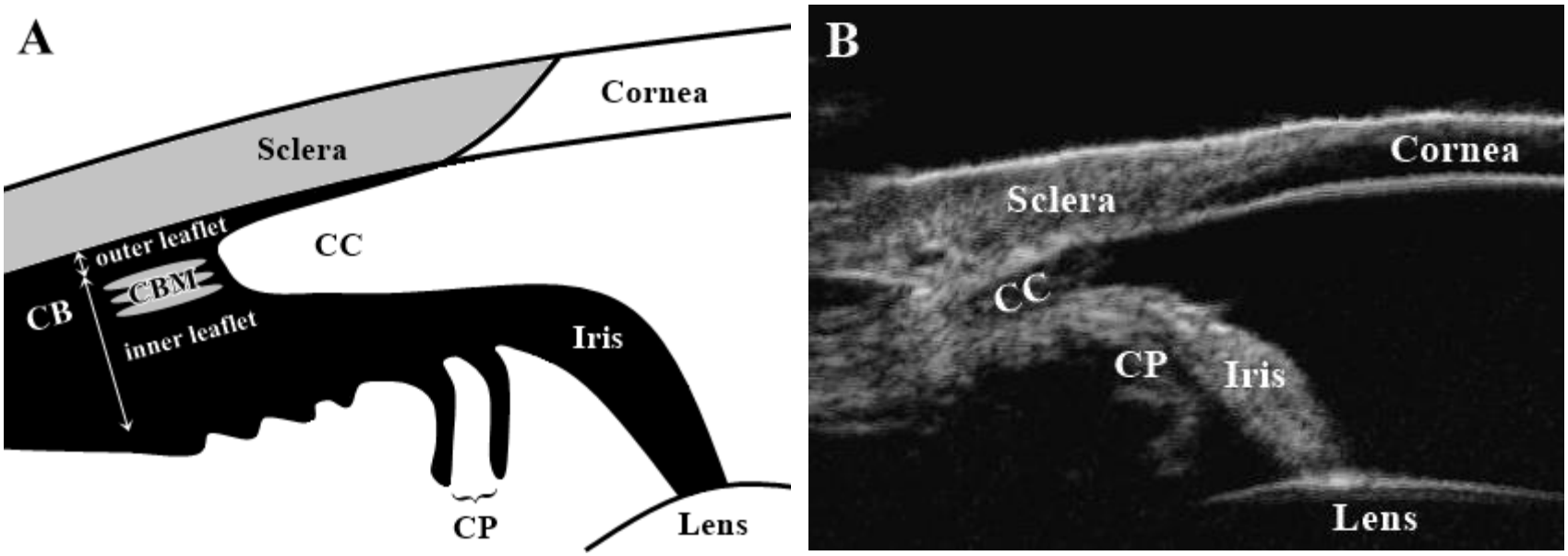

Figure 8. Schematic diagram (A) and representative UBM image (B) of the iridocorneal angle and CC region in a dog. The ciliary body (CB) splits into 2 portions to form the CC. The ciliary body musculature (CBM) of the outer leaflet is much smaller than that of the inner leaflet (the inner leaflet forms the inner border of the CC). CP = Ciliary process.

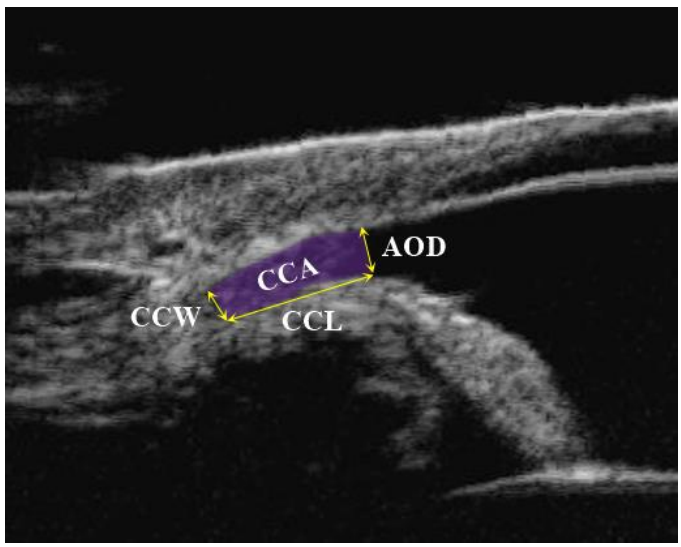

Figure 9. An enlarged version of the representative UBM image in Figure 8. Measurement of the AOD, CCW, CCL, and CCA is indicated.

UBM = ultrasound biomicroscopy; AOD = angle opening distance (distance on a perpendicular line from the surface of the peripheral iris root to the inner surface of the cornea or sclera); CCW = posterior width of ciliary cleft (distance from the internal border of the outer leaflet of the ciliary body to the internal border of the inner leaflet of the ciliary body at the caudal end of the ciliary cleft); CCL = ciliary cleft length (distance between the caudal end of the ciliary cleft and the surface of the peripheral iris root); CCA = ciliary cleft area (cleft area surrounded by the internal border of the ciliary cleft and AOD)

4. Statistical analysis

Mean and standard deviations (SD) values were calculated for pupil diameter, IOP, and UBM measurements in each eye of each dog for each phase. Differences for the control eyes among the 4 phases were evaluated by use of the Kruskal-Wallis test. Within each phase, results for drug-treated eyes were compared with results for control eyes by use of the Wilcoxon signed rank test. Differences for each variable of the drug-treated eyes among the 4 phases were evaluated by use of the Kruskal-Wallis test. Statistical analyses were performed with commercially available software (SPSS, version 21, SPSS Inc, Chicago, Ill). Values of $P < 0.05$ were considered significant.

Results

Mean ± SD pupil diameter of the control eyes was 6.7 ± 1.2 mm, 7.4 ± 0.9 mm, 7.0 ± 0.6 mm, and 7.4 ± 0.9 mm for phases L, P, PL, and LP, respectively; values did not differ significantly among the 4 phases. Mean ± SD pupil diameter of drug-treated eyes was 1.1 ± 0.2 mm, 1.4 ± 0.5 mm, 1.0 ± 0.0 mm, and 1.0 ± 0.0 mm for phases L, P, PL, and LP, respectively. All drug-treated eyes had marked miosis; however, pupil diameter of the drug-treated eyes did not differ significantly among the 4 phases.

The IOPs for the control and drug-treated eyes were determined for all phases (Table 3). No significant differences were identified for the control eyes among the 4 phases. Similarly, no significant differences were identified for the drug-treated eyes among the 4 phases. However, mean IOPs of the drug-treated eyes were significantly lower, compared with the mean IOPs of the control eyes, for all phases, except for phase P.

Table 3. Mean ± SD values of IOP measured in eyes of 6 dogs during each of 4 phases.

	IOP (mmHg)		
	Control eyes	Treated eyes	ΔIOP
Phase L	14.89 ± 2.44	12.67 ± 3.52*	2.22 ± 1.89 (14.9%)
Phase P	13.22 ± 2.47	11.33 ± 2.44	1.89 ± 3.61 (14.3%)
Phase PL	11.72 ± 2.47	8.67 ± 1.74*	3.06 ± 2.59 (26.1%)
Phase LP	12.39 ± 2.52	9.22 ± 0.54*	3.17 ± 2.54 (25.6%)

Latanoprost (phase L), pilocarpine (phase P), pilocarpine followed 5 minutes later by latanoprost (phase PL), and latanoprost followed 5 minutes later by pilocarpine (phase LP) were administered to the right eye of each dog. Artificial tears (placebo treatment) were administered to the left eye (control eye) during each phase. Washout period between treatments was ≥ 5 days.

*Within a phase, the value differs significantly ($P < 0.05$) from the value for the control eye.

SD = standard deviations; IOP = intraocular pressure; ΔIOP = Difference in IOP between control and drug-treated eyes.

Values of all UBM variables were determined for all 4 phases (Table 4). The AOD and CCA were significantly higher in the drug-treated eyes than in control eyes for all phases. The CCW was significantly higher in the drug-treated eyes than in the control eyes for phases P, PL, and LP. The CCL was significantly higher in the drug-treated eyes than in the control eyes only in phases PL and LP.

Table 4. Mean ± SD values of UBM variables measured in eyes of 6 dogs during each of 4 phases.

	AOD (mm)	CCW (mm)	CCL (mm)	CCA (mm^2)
Phase L				
Control	0.36 ± 0.10	0.27 ± 0.07	1.93 ± 0.21	0.70 ± 0.17
Drug-treated	0.55 ± 0.04*	0.24 ± 0.03	2.03 ± 0.20	0.92 ± 0.14*
Phase P				
Control	0.35 ± 0.08	0.29 ± 0.05	1.58 ± 0.43	0.58 ± 0.16
Drug-treated	0.60 ± 0.08*	0.44 ± 0.09*	1.64 ± 0.31	0.95 ± 0.22*
Phase PL				
Control	0.36 ± 0.09	0.27 ± 0.08	1.46 ± 0.23	0.53 ± 0.21
Drug-treated	0.59 ± 0.08*	0.41 ± 0.08*	1.88 ± 0.41*	0.96 ± 0.21*
Phase LP				
Control	0.39 ± 0.06	0.28 ± 0.08	1.70 ± 0.24	0.63 ± 0.14
Drug-treated	0.57 ± 0.04*	0.34 ± 0.09*	1.83 ± 0.21*	0.89 ± 0.16*

*Within a variable within a phase, value differs significantly ($P < 0.05$) from the value for the control eye.

Latanoprost (phase L), pilocarpine (phase P), pilocarpine followed by latanoprost (phase PL) and latanoprost followed by pilocarpine (phase LP) were administered to the drug-treated eye and artificial tears were administered to the control eye. SD = standard deviations; UBM = ultrasound biomicroscopy; AOD = angle opening distance; CCW = posterior width of ciliary cleft; CCL = ciliary cleft length; CCA = ciliary cleft area.

Comparison of results for drug-treated eyes among the 4 phases revealed that the CCW differed significantly between phases L and P and between phases L and PL (Figure 10). Other variables for the drug-treated eyes did not differ significantly among the phases.

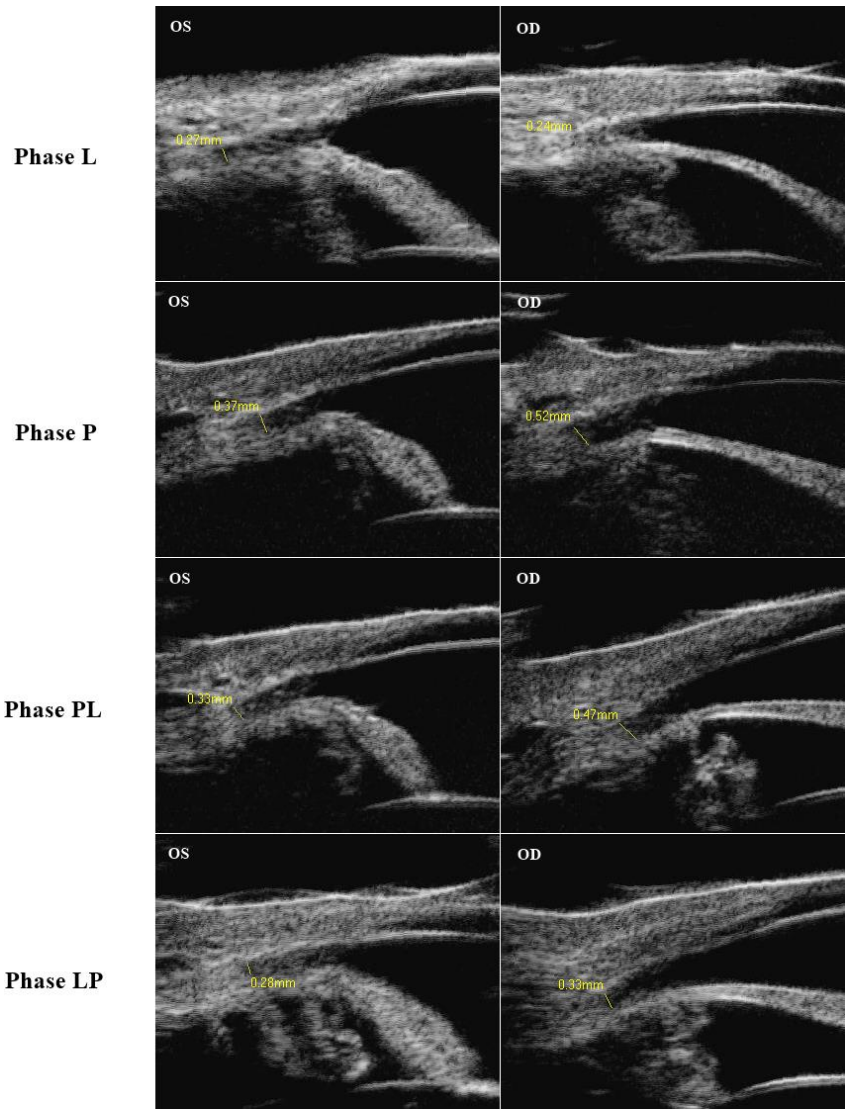

Figure 10. Representative images measuring the CCW (posterior width of ciliary cleft) in the same dog. Latanoprost (phase L), pilocarpine (phase P), pilocarpine followed by latanoprost (phase PL) and latanoprost followed by pilocarpine (phase LP) were administered to the right eye (OD) and artificial tears were administered to the left eye (OS). The CCW of treated eye in phase P was wider than that in phase L. When used in combination, CCW of treated eye in phase PL was wider than that in phase LP. The CCW of treated eye in phase LP was similar to that in phase L.

Discussion

In the study reported here, administration of both pilocarpine and latanoprost induced marked miosis in the eyes of dogs. Miosis has been described as an important side effect caused by prostaglandin analogues in several studies of dogs (Sarchahi et al., 2012; Plummer et al., 2013; Yoshitomi and Ito, 1988). Although pilocarpine as a cholinergic agonist is considered to be a traditional miotic agent (Sarchahi et al., 2012; Plummer et al., 2013), latanoprost-induced miosis was not significantly different from pilocarpine-induced miosis. There was no significant difference in pupil size among prostaglandin-mediated miosis, cholinergic-mediated miosis, and miosis caused by concurrent administration of both drugs in the present study.

Several UBM variables were used as indicators of iris or ciliary body movement to describe changes in the anterior segment after administration of the 2 classes of miotic agents. The increase in AOD and CCW were used as surrogate indicators of centripetal displacement of the iris root and inner leaflet of the ciliary body, respectively. The increase in CCL was considered to indicate axially oriented expansion of the CC likely attributable to rostral or caudal (or both) movement of the inner leaflet of the ciliary body.

Both pilocarpine and latanoprost expanded the cleft area to a similar degree, which appeared to be the result of centripetal traction of the iris root represented as the increase in AOD. The iris root was also used as one of the landmarks of CCL; however, the CCL did not differ significantly between the control and drug-treated

eyes in phases L and P. This might imply that the inner leaflet of the ciliary body connected to the iris root did not stretch or move rostrally despite the latanoprost-induced and pilocarpine-induced miosis. The pectinate ligament apparently acts as a strut to prevent excessive widening and stretching of the cleft (Gum et al., 1993; Morrison and Van Buskirk, 1982). However, significant increases in the CCL were identified in phases PL and LP (ie, when both drugs were used in combination). These changes appeared to be clinically unimportant, considering that the values of the drug-treated eyes in phases PL and LP were well within the range of measurements for control eyes across the 4 phases.

Latanoprost alone in phase L and the combination of both drugs in phases PL and LP induced a significant decrease in IOP. Although AOD and CCA were significantly increased after miosis in all 4 phases, it is unclear whether these changes were directly related to IOP reductions because pilocarpine alone (phase P) did not cause a significant reduction in IOP (Sarchahi et al., 2012; Gum et al., 1993). In other words, we were unable to establish a causative link between CC configurations and IOP reductions in the present study. Further studies are needed to clarify the role of miosis and resultant CC expansion in the reduction of IOP. Clinically, dramatic IOP reduction was achieved by latanoprost-induced miosis in dogs with primary closed-angle glaucoma, which was attributable to reduced iridolenticular contact at the pupillary margin by miosis (Miller et al., 2003). Because pilocarpine also induces miosis but does not decrease IOP as profoundly as latanoprost, despite their similar degree of CC expansion, we sought to identify differences in anterior ocular segments between prostaglandin-mediated and

cholinergic-mediated miosis to provide evidence of another mechanism of action for reducing IOP.

An important finding in the study reported here was that the CCW differed between the 2 classes of miotics and also on the basis of the order of administration of those miotics. Although latanoprost alone did not modify the CCW, and even slightly decreased it in phase L, pilocarpine induced a significant increase in the CCW in phase P. Considering that the smooth muscle bundles of the outer leaflet of the ciliary body are much smaller than those of the inner leaflet of the ciliary body (Samuelson and Streit, 2012) and that the inner leaflet of the ciliary body forms the inner border of the CC (Carreras et al., 1997), the CCW could have been affected by the musculature of the inner leaflet of the ciliary body.

Latanoprost and pilocarpine have opposing actions on ciliary muscle contractility (Toris et al., 2008; Toris et al., 2001; Grierson et al., 1978, Toris et al., 2002; Plummer et al., 2013). In humans and other primates, latanoprost causes short-term relaxation of ciliary muscle bundles (Toris et al., 2008; Toris et al., 2001; Toris et al., 2002). Although the effect of latanoprost on ciliary muscle contractility of dogs has not yet been determined, it was suggested in a recent UBM study (Park et al., 2016) of dogs that a similar short-term relaxation is probable. In the present study, relaxation of the ciliary muscle 2 hours after latanoprost administration and the associated decrease in ciliary body thickness (similar to that reported in another study (Park et al., 2016)) may have caused the nonsignificant reduction in CCW. On the other hand, pilocarpine is a cholinergic agent that causes contraction of

ciliary muscle bundles (Toris et al., 2001, Grierson et al., 1978; Toris et al., 2002; Kent et al., 1999), which could have caused a thickening of the inner leaflet of the ciliary body musculature and led to the increase in CCW identified in the present study. In addition, contraction of the ciliary muscle by pilocarpine can expand the anterior lamellated trabecular meshwork, which is equivalent to the CC in dogs (Carreras et al., 1997; Overby et al., 2014). This also could have contributed to the increase in CCW.

Evaluation of the order of administration of pilocarpine and latanoprost (phases PL and LP) revealed contradictory results. Although significant increases in CCW were identified in drug-treated eyes in phases PL and LP, compared with results for the control eyes, the increase of the CCW in phase LP was less than that in phase PL. In addition, the CCW of phase LP was not significantly different from the CCW of phase L. On the basis of results of the present study, the order of pilocarpine and latanoprost administration affected ciliary muscle contractility such that the first drug determined the ciliary muscle contractility and associated cleft morphology, which was not fully reversed by the second drug.

Clinically, the progressive and irreversible closure of the CC is a main feature of primary closed-angle glaucoma in dogs (Plummer et al., 2013; Miller and Bentley, 2015). Because the CC of most glaucomatous dogs is already collapsed at the time of the initial examination, drugs that increase the CCW may not be able to reopen the CC; however, it may be worthwhile to administer such drugs because even minimal alterations in the CCW can improve aqueous humor outflow. Dogs for

which the iridocorneal angle is partially occluded by pectinate ligament dysplasia or dogs that have repeated episodes of intermittent angle closure could benefit from a treatment regimen targeted to widen the CC. However, it should be mentioned that the SDs for the CCW were quite large (especially in phases PL and LP) in the present study. Because it sometimes was difficult to distinguish the caudal margin of the CC from echogenic fibrillar strands located inside the CC, and because the size of the built-in caliper cursor was quite large, there could have been some error in measurement of the CCW.

Latanoprost is used as the sole agent or in conjunction with other topical hypotensive drugs to treat dogs with glaucoma (Alario et al., 2015). However, administration of latanoprost in combination with pilocarpine did not result in a significant difference in IOP from administration of latanoprost alone in the present study. There was a nonsignificant additional reduction in IOP identified when both drugs were administered together. Further studies are necessary to determine the additive effects of pilocarpine and latanoprost in dogs.

Results of the present study might indicate that anterior segment morphology is not directly related to reductions in IOP. In addition, it is possible that the hypotensive action of latanoprost could not be attributed solely to enhancement of uveoscleral outflow. There is evidence that prostaglandin derivatives could also increase conventional trabecular outflow and reduce aqueous production in dogs (Toris et al., 2008; Taniguchi et al., 1996; Richter et al., 2003; Takagi et al., 2004; Ward, 2005). However, results of a more recent study (Fentiman et al., 2017)

suggest that latanoprost does not alter aqueous humor production in dogs. The complexity of the mechanism of action of prostaglandin could be another reason that gross anatomic changes identified in the present study did not fully explain reductions in IOP.

The study reported here had several limitations. The small number of dogs used in the present study could have been a major reason that significant differences were not detected. The response rate to each drug must also be considered (Miller, 2013). There was 1 dog that did not respond to latanoprost and 2 dogs that did not respond to pilocarpine. This could have restricted our ability to detect differences between effects of pilocarpine and latanoprost. Moreover, it is also possible that the timing of IOP measurements in the present study did not reflect the maximal ocular hypotensive effects of the drugs. In dogs, the maximal IOP-lowering effects of 2% pilocarpine and latanoprost are evident at 3 and 5 to 6 hours after administration of a single dose, respectively (Gwin et al., 1977; Kahane et al., 2016; Smith et al., 2010). Additionally, over time, latanoprost enhances uveoscleral outflow through remodeling of the extracellular matrix between ciliary muscle bundles, reaching maximal reduction of IOP the fifth day of twice-daily administration to dogs (Sarchahi et al., 2012). This chronic, hypotensive effect of latanoprost could not occur within 2 hours after administration of a single dose. Because most glaucomatous patients would likely receive once-daily (humans) or twice-daily (dogs) administration of latanoprost for an extended period, a multiple-dose study with a treatment duration of at least 5 days is needed to elucidate the relationship between structural changes and reductions in IOP.

Conclusions

Prostaglandin-mediated and cholinergic-mediated miosis in dogs resulted in differences in CC morphology that were related to the contractility of the ciliary musculature. When both drugs were administered in combination, the first drug administered determined the cleft morphology, which was not fully reversed by the second drug.

GENERAL CONCLUSIONS

UBM allowed anterior ocular segment observation at near microscopic resolution in small birds like pigeons in Chapter I of this study. However, resolution of UBM equipment was insufficient to clearly differentiate the angular aqueous plexus from the ciliary cleft in pigeons. Since UBM was well tolerated in pigeons without the use of any sedative or anesthetic, UBM could be widely utilized in the entire field of veterinary ophthalmology.

UBM was also useful to visualize structural changes induced by topical latanoprost. In Chapter II of the present study, the effects of topical latanoprost on the anterior segment and ciliary body were evaluated. It was demonstrated that the early and the long-term effects of latanoprost on the uveoscleral outflow pathway could occur in dogs as in humans and primates. Also, these effects were suggested to be the result of ciliary muscle relaxation and extracellular matrix remodeling by topical latanoprost.

To reveal the role of miosis in reducing IOP and to compare prostaglandin-mediated and cholinergic-mediated miosis in dogs, UBM was performed after topical administration of latanoprost and pilocarpine in Chapter III. Despite the similar degree of miosis between prostaglandin-mediated and cholinergic-mediated miosis, the posterior width of CC was wider when pilocarpine induced ciliary muscle contraction and cholinergic miosis. When both miotics were used in combination, the order of administration affected ciliary muscle contractility such that the first drug determined the ciliary muscle contractility and associated cleft

morphology. Since aqueous outflow resistance could be influenced by the ciliary cleft width, the findings of the present study should be considered in medical managements of canine glaucoma.

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국 문 초 록

정상 비둘기와 녹내장 안약을 투여한 개에서 초음파 생체현미경을 이용한 전안부 분석

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본 연구는 초음파 생체현미경을 활용하여 비둘기와 개의 전안부 구조를 분석하였다.

제 1장에서는 비둘기에서 초음파 생체현미경 검사를 실시하여 정상 비둘기 안구 전안부 구조물의 여러 계측치를 제시하였다. 10마리의 정상안을 가진 비둘기에서 초음파 생체현미경 검사를 실시하였다. 모양체 틈새의 길이, 너비, 면적 및 홍채각막각의 각도를 측정하였다.

이들의 평균값은 각각 1.55 ± 0.17 mm, 0.36 ± 0.05 mm, 0.39 ± 0.04 mm^2, 15.17 ± 1.06° 로 확인되었다.

제 2장에서는, latanoprost 점안액이 개에서 안압을 하강시키는 작동 기전을 밝히기 위해, 6마리의 비글견에서 latanoprost 점안 후 안구 전안부와 모양체의 구조적 변화를 초음파 생체현미경을 이용하여 분석하였다. Latanoprost 점안 전과 점안 2시간 후에 초음파 생체현미경 검사를 실시하였으며, 이후 1주일 동안 하루에 2번씩 latanoprost를 점안한 후에 다시 한번 초음파 생체현미경 검사를 실시하였다. 홍채각막각의 각도, 모양체 틈새 입구의 너비, 모양체 틈새 중간부 너비, 모양체 틈새의 길이, 모양체 두께를 측정하여 각 시기별로 비교 분석하였다. 그 결과 latanoprost는 홍채각막각과 모양체 틈새 입구의 너비를 감소시켰고, 모양체 틈새 중간부 너비는 증가시켰다. 모양체 두께는 점안 2시간 후에는 감소하였고, 점안 1주일 후에는 증가하였다. 이러한 결과는 개에서도 latanoprost 점안에 의한 포도막공막 방수 유출 증진 효과가 있음을 짐작케 한다.

제 3장에서는 안압 하강에 있어 축동의 역할을 비교 분석하기 위해 프로스타글란딘 유래 축동을 유도하는 latanoprost 점안액과 콜린성 축동을 유도하는 pilocarpine 점안액을 단독 혹은 병용 점안 후 초음파 생체현미경 검사를 실시하였다. 프로스타글란딘 유래 축동과 콜린성 축동의 경우 동공 직경에 있어서는 유의적인 차이가 없었지만, 모양체

틈새 후방 너비가 각 점안액이 유발하는 모양체 근육의 수축도에 따라 결정됨을 알 수 있었다. 이에 따라 콜린성 축동이 프로스타글란딘 유래 축동에 비해 모양체 틈새 후방 너비를 유의적으로 증가시켰다. 또한 병용 점안 시에는 latanoprost와 pilocarpine의 점안 순서에 의해서도 모양체 틈새 후방 너비가 유의적으로 달라짐을 밝혔다.

본 연구 결과, 초음파 생체현미경은 소형 조류와 개의 전안부 구조를 평가하는 유용한 도구임이 입증되었다. 특히, 초음파 생체현미경을 활용하여 latanoprost 점안액이 개에서 포도막공막 방수 유출을 증진시킨다는 것을 간접적으로 증명하였다. 또한 latanoprost 점안액에 의한 프로스타글란딘 유래 축동과 pilocarpine 점안액에 의한 콜린성 축동은 모양체 틈새의 모양을 다르게 변화시킨다는 점을 밝혔다. 모양체 틈새의 너비는 방수 유출 저항과 밀접한 관련이 있으므로 본 연구의 결과는 개 녹내장의 약물 치료에 있어 고려되어야 할 것이다.

주요어: 개, latanoprost, 모양체 틈새, 비둘기, 초음파 생체현미경, pilocarpine

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