Effects of oral gabapentin, tramadol and meloxicam on ocular parameters in healthy dogs

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ABSTRACT

EFFECTS OF ORAL GABAPENTIN, TRAMADOL AND MELOXICAM ON OCULAR PARAMETERS IN HEALTHY DOGS

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The objectives of this study were to determine the effects of oral gabapentin, tramadol and meloxicam on ocular parameters when given to healthy dogs for three days. Nine, healthy, intact male Beagles were utilized for a randomized, blinded, case-crossover study using a 6-sequence, 3-treatment, 3-period design, including a 7-day acclimation period, three, 3-day treatment periods followed by a 7-day washout period. Dogs received gabapentin (10mg/kg PO q8hr), tramadol (3mg/kg PO q8hr), and meloxicam (0.2mg/kg PO once, then 0.1mg/kg PO q24). Ocular parameters were evaluated several times per day.

Comparing acclimation period measurements from days 1-5 and 6-7 confirmed that a 5-day acclimation period is required for intraocular pressure values to return to baseline in canine patients (p-value = 0.0021).

When accounting for repeat measures within individuals, gabapentin and tramadol significantly affected only IOP (-0.45 and -0.35mmHg, respectively; p-value = 0.038). Although statistically significant, this reduction is not considered clinically significant.

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LIST OF ABBREVIATIONS

AHL Animal Health Laboratory

AP Acclimation period

CAF Central Animal Facility

CBC Complete blood count

CI Confidence interval

COX Cyclooxygenase

CTT Corneal touch threshold measured in cm

GPE General physical examination

IOP Intraocular pressure measured in mmHg

LD Loading dose

LPS Lipopolysaccharide

NSAID Non-steroidal anti-inflammatory drug

OVC Ontario Veterinary College

PD Pupillary diameter measured in mm

PI Primary investigator

PO Per os

STT Schirmer tear test measured in mm/min

TBUT Tear break-up time measured in seconds

Tx Treatment

WO Washout period

CHAPTER 1: LITERATURE REVIEW

1.1 Introduction

The purpose of this literature review is to provide an overview of the ophthalmic tests frequently utilized in small animal veterinary practice to evaluate ocular health. It will discuss their relevancy, and potential limitations in a clinical and research setting, and will summarize the available research evaluating the effects of three oral analgesics on ophthalmic health. These analgesics, namely gabapentin, tramadol and meloxicam, are commonly prescribed in veterinary medicine to treat pain in canine patients and little is known of their potential relevancy regarding ocular health. This review will clearly outline existing gaps in current veterinary research and will provide justification to undertake further study of long-term analgesic use of gabapentin, tramadol and meloxicam, and their potential effects and impact on ocular health.

1.1.1 Background

Treatment of chronic and acute pain in canine patients is a daily occurrence in veterinary medicine. Several different classes of analgesics may be used to achieve effective pain relief in the dog and may be used individually or in combination. Recognized conditions requiring pain relief include, but are not limited to: ocular disease, orthopedic disease, traumatic wounds, gastrointestinal disease, inflammatory conditions, pain secondary to neoplasia, and treatment of patients pre-, intra-, and post-operatively. Lack of adequate pain control can result in both physical (e.g. alterations in normal vital parameters, decreased appetite, activity) and psychological trauma (e.g.

reactivity in response to an anticipated painful stimuli) to canine patients. Consequently, following release from a veterinary hospital, it is common practice for a canine patient to be discharged with a recommendation for ongoing administration of analgesics at home, often for several days. Canine patients may be prescribed a variety of analgesics including opioids, neuropathic pain analgesics and non-steroidal anti-inflammatory drugs (NSAIDs)[1]. Despite analgesics being commonly used and prescribed in small animal practice, to the author's knowledge, there are few, if any, studies evaluating the effects of systemic analgesics on ocular parameters in canine patients when administered over several days. More specifically, there are no studies evaluating the ocular effects of repeated doses of orally administered gabapentin, tramadol and meloxicam.

1.1.2 Analgesia and Relevance Regarding Ocular Health

External and internal factors that decrease tear production, alter intraocular pressure (IOP), alter pupil size, expedite tear break-up time (TBUT) and decrease corneal sensitivity, may predispose a canine patient to the development of ocular pathology, or worsen existing ocular disease[2]. This potential impact is increased in patients with breed predispositions to ocular conditions or diseases (e.g. brachycephalic canine patients, and recognized breeds with heritable ocular disorders), and those suffering from certain systemic diseases (e.g. endocrinopathies)[3–5].

A potential benefit of administration of analgesia is that it may provide pain relief and decrease inflammation to the eye (e.g. in cases of uveitis and corneal trauma). It is

important to evaluate whether the prescribed analgesic provides an appreciable benefit to the ocular condition being treated; however, it is also critical to evaluate whether the analgesic has a detrimental effect on the eye. To provide a true overview of the effect a medication may have on ocular health, a comprehensive ophthalmic evaluation should be performed, including the effect a medication has on tear production, intraocular pressure (IOP), pupillary diameter (PD), tear film stability and corneal sensitivity. For example, a medication that decreases tear production or destabilizes tear film integrity may predispose a patient to development of ulcerative keratitis. Similarly, any medication that increases IOP may worsen existing glaucoma, or act as a catalyst in a patient genetically predisposed to development of glaucoma. Medications that decrease corneal sensitivity may result in a stunted blink reflex, thereby increasing a patient's risk of corneal trauma.

Available studies evaluating the effect of sedatives, analgesics and general anesthesia on ocular parameters have evaluated only one or two measures of ophthalmic testing, and have performed those evaluations only over a 24-48 hour time period maximum[4,6–12]. As most recognized painful conditions require analgesia for several days, further research into the effects of longer term administration of analgesics on canine ocular parameters is warranted.

1.2 Ocular Examination Techniques

1.2.1 Introduction

A complete ophthalmic examination typically includes a neuro-ophthalmic examination, Schirmer Tear Test I (STT I), corneal fluorescein stain uptake, tonometry using a

handheld tonometer, slit-lamp biomicroscopy, and direct and indirect ophthalmoscopy[8,13–16].

Schirmer tear test I, corneal fluorescein stain uptake, and evaluation of IOP are non-invasive tests that are frequently performed on canine patients in general practice. The STT I remains the gold standard for measuring tear production in canine patients. Fluorescein stain uptake is performed to evaluate corneal integrity and to rule out the presence of corneal ulcers. Several handheld tonometers are available in clinical practice providing an estimation of IOP in canine patients. Additional ophthalmic tests that should be performed to ensure maintenance of ocular health include evaluation of pupillary diameter (a component of the neuro-ophthalmic examination), tear film stability, which is established by measuring tear break-up time (TBUT; evaluated with the use of the fluorescein stain), and corneal sensitivity which is established by evaluating corneal touch threshold (CTT).

Schirmer tear test I, IOP, PD, TBUT and CTT will be reviewed in detail and their relevancy with regards to evaluating ocular health, both in a clinical and a research setting, will be discussed.

1.2.2 Tear Production and Schirmer Tear Test

1.2.2.1 History and Methods of Testing

The preocular tear film is made up of three distinct and vital layers, including the outer lipid layer, middle aqueous layer, and innermost mucin layer[2]. Tears are uniformly and consistently spread over the surface of the cornea via blinking[2]. This preocular tear film

is responsible, amongst other functions, for removing ocular debris, ensuring a uniform corneal appearance, and providing nutrients, vital elements and immunologic defense for the eye[2]. Insufficient tear production is referred to as keratoconjunctivitis sicca (KCS) or “dry eye”, which may be temporary or permanent[2]. Keratoconjunctivitis sicca is a well-documented condition in canine patients and maintaining adequate tear production is vital to ensure ocular health and for maintenance of corneal integrity[2]. Insufficient quantity or quality of the tear film can predispose a patient to development of ulcerative keratitis, causing significant pain to the patient and may threaten vision[17,18].

Tear production, which includes both basal and reflex tears, may be measured quantitatively and qualitatively in canine patients[2]. A quantitative measure of tear production is established via Schirmer tear tests (STT) I and II, whereas qualitative tear production is measured by evaluating tear break-up time (TBUT) (discussed in a subsequent section)[2]. The STT was introduced by Otto Schirmer in 1903[2,19,20], and first described in the dog in 1962[21]. Schirmer tear test I, which measures the aqueous component of the tear film, is most commonly performed in clinical practice. It provides an estimation of both basal and reflex tear production and is influenced by the amount of tears existing in the conjunctival sac prior to the test being performed[22]; STT II measures basal tear production only[2,23]. Schirmer tear test II results are less applicable for the normal physiological status of a patient, and more accurately represent tear production in anesthetized individuals[24].

Schirmer tear test I is accomplished by inserting a standardized strip of specialized paper[a] into the ventral conjunctival sac for one minute, without the application of topical

anesthesia. The amount of wetting to the paper is recorded in mm/min, with quoted values of 18.89+/-2.62 and 21.0+/-4.2mm/min considered normal for a dog[7,23,25]. It is always recommended to evaluate the STT I in both eyes concurrently to ensure that lacrimation in both eyes is within normal limits[18]. In human patients, STT I is performed over a five minute period[19]; however, this length of time is not feasible in the typical, awake canine patient and has resulted in modification of this test in veterinary medicine. The STT II test is performed by administering topical anesthesia to the eye (thereby preventing reflexive tearing), removing fluid from the ventral conjunctiva with a cotton applicator, and an STT I strip is the inserted into the ventral conjunctiva as described above with normally quoted values for the dog between 3.8+/- 2.7 and 11.6+/- 6.1mm/min[2,25,26].

The age of a patient at the time of testing has also been shown to have an impact on tear production. Both basal and reflexive tear production have been documented to increase during the first 9-10 weeks of life in the dog with neonates having a lower tear production versus adult canines[25]. This 2014 study performed by Verboven *et al*. evaluated tear production during the morning hours only when tear production is lower in adult canines. A separate study performed by Hartley *et al.* documented that tear production decreases with age in normal adult canine patients, calculated at 0.4mm/min decrease for every one year of increase in age[26]. In contrast to the 2006 Hartley study, studies performed by Komnenou *et al.* and Chandler *et al.* did not confirm any age-related changes in STT I values[27,28].

A 1998 study conducted by Berger and King found that for every 0.45kg increase in body weight, there was a corresponding 0.02mm/min increase in STT I values, and a 0.12mm/min increase in STT II values prompting the hypothesis that this could be the reason that smaller dogs are at a higher risk for development of KCS; however, there were various limitations with this study including that STT I and II values were measured once per day only, and the same population of dogs was not utilized for both studies[29]. Furthermore, other authors have determined that STT I values did not appear to be impacted by body weight[25,30].

Studies performed by Verboven *et al.* and Soontornvipart *et al*. found that there was no significant difference in STT I or II values between sexes[25,30], and the absence of gender difference in STT I results was also confirmed by Komnenou *et al*., and Chandler *et al.*[27,28,30].

1.2.2.2 Limitations and Available Research Studies

Despite its widespread use in small animal clinical practice, several studies have documented challenges and limitations associated with the STT I[19,26,31–33]. Potential limitations associated with measurement of tear production include: environmental factors, location of the tear test strip and eye position, patient-associated factors, diurnal variation, variability between testing paper lot numbers, and the concurrent use of medications.

A 1967 study performed by Williamson on human patients demonstrated 20% of patients exposed to higher temperatures and lower humidity values had lower STT I

values than patients exposed to higher humidity environments and lower temperatures; however, statistical significance of these findings was not elucidated[31]. A study analyzing a questionnaire provided to office workers in Glasgow, Scotland, also identified that several variables exist that could lead to ocular irritation impacting tear production, including exposure to allergens, building heating and cooling practices, and air flow[34]; however, the questionnaire relied on the honesty of respondents and did not take into account potential variables related to respondent lifestyle. A subsequent study in human patients evaluated whether eye position would have an effect on tear production[35]. This study found that an inferior eye position resulted in a higher STT I value when compared to primary and superior eye positions and suggested that in humans, the eye position including whether the eye was open or closed, should be noted to ensure repeatability in results[35]. Location of tear test strip placement is also significant, as placement of the strip in the dorsal conjunctiva will result in a significantly lower STT I value versus when placed in the ventral conjunctiva[36]. Stipulating the eye position of an awake canine patient would be extremely problematic, as the clinician cannot ask a canine patient to direct their eye a certain way, thus it is unknown whether eye position has a significant impact on STT I values in dogs. There is, therefore, the potential for systematic error when performing the test itself due to operator error including variability in where the strip is placed in the conjunctival fornix, presence of digital pressure around the eye, and difficulty and lack of accurate repeatability when inserting the strip. The knowledge that eye position and tear strip placement may impact STT I results should make the clinician cognizant of the requirement to ensure

consistent and correct placement of the tear test strip at every evaluation time for reproducible and accurate results.

Canine patient-associated factors have also been implicated in affecting STT I values, including the presence of systemic illness and stress levels at the time of testing. STT I has also been suggested to be impacted by the breed of dog tested. A 2013 study performed by Chandler *et al.* evaluated the STT I values of dogs hospitalized in the intensive care unit suffering from a multitude of systemic illnesses including: heart disease/heart failure, kidney disease, endocrinopathies, gastrointestinal disease, and many other diseases[28]. STT I results from hospitalized dogs were compared to control dogs free of ocular and systemic disease[28]. Ultimately, regardless of the underlying systemic illness, this study concluded that the presence of general systemic illness resulted in a decrease in tear production in canine patients[28]. It should be noted that this study evaluated patients who were receiving a variety of systemic medications, including analgesics and sedatives, which would have impacted their STT I results. A patient's demeanor at the time of testing may also impact their results with some authors suggesting that an increase in sympathetic tone may increase lacrimation[24], and anticipating tear strip placement may cause reflexive lacrimation[29]. Hamor *et al.* reported a statistically significant difference in mean STT results (p-value ≤ 0.05) when comparing five different purebred dog breeds, including Beagles, Labrador Retrievers, English Springer Spaniels, Shetland Sheepdogs and Golden Retrievers[37], where Shetland Sheepdogs had a decreased tear production when compared to all other breeds evaluated (p-value < 0.001). However, researchers in this study evaluated STT I

values once on client owned dogs (all breeds listed except for the Beagles), whereas the Beagles were evaluated twice daily for five days[37]. This difference in the number of data points collected may not have provided a fair comparison between the research colony Beagles. Data points were also collected at different times of day[37], which may have confounded results due to previously documented diurnal changes in tear production. Ultimately, STT I results require the clinician to critically interpret values obtained in light of patient-specific factors, including presence or absence of systemic illness, and their demeanor at the time of testing.

The effect of circadian rhythm on STT I values has been well documented in veterinary medicine, with tear production noted to display a nocturnal peak[26,33] with a documented 0.7mm increase at 4PM when compared to values obtained at 10AM (p-value = 0.04)[26]. Berger and King noted a weekly fluctuation in STT I values in their 1998 study[29]; however, their study did not provide specific values, nor did they include their statistical methodology in support of their statements[29]. The 2009 study performed by Giannetto *et al.* demonstrated lowest STT I values at 8AM, with highest values obtained at 8PM[33]. Their documented variation in STT I value was a quoted statistically significant mean of 2.31mm/min lower in the morning versus the evening; however, the authors speculated that this difference was not likely to be significant clinically, and no p-value was included in the paper[33].

It is also accepted that tear production values can be impacted by inherent variability in manufacturer lot numbers and the type of test strips used[25,33,38]. A 2018 study evaluating five different tear test strips found a statistically significant difference (p-value

= 0.001) in the uptake of tears between different test strips, secondary to strip construction and the lack of standardization of commercially available tear test strips[38]. For this reason, it is imperative that the examiner note the lot number of the testing strips used, especially when used in a research setting.

Numerous commonly used medications, including sedatives, analgesics, parasympatholytics, and general anesthetics have also been shown to decrease tear production in canine patients[4,7,9,12,27,30,39–41]. The decrease is hypothesized to be secondary to a decrease in the autonomic pathways responsible for tear production[4]. A 2009 study conducted by Sanchez *et al.*, evaluated the effects of systemically administered medetomidine and medetomidine and butorphanol given in combination, on STT I values in client owned dogs[6]. The study demonstrated that tear production was significantly decreased 15 minutes post-sedation administration compared to baseline, and that “in most cases” STT I values returned to normal following administration of the reversal agent atipamezole[6]. This study evaluated STT I values in isolation, without evaluating other parameters of ocular health and did so for a short period of time (minutes following administration only). Similarly, a 2011 study conducted by Ghaffari *et al.* studied the effects of chlorpromazine alone and chlorpromazine and morphine given as a combination on STT I values in canine patients free of ocular disease[7]. Results of this study showed that tear production was significantly decreased in both groups after one and two hours; however, the researchers did not evaluate STT I results beyond the two hour mark[7]. A short communication submitted by Biricik *et al.* in 2004 evaluated the effects of 5mg/kg pethidine or 10mcg/kg fentanyl on STT I values in

clinically healthy dogs[9]. Both drugs were noted to result in a decrease of STT I values, but effects were not evaluated beyond the 20 minute mark[9]. Changes in STT I values were also evaluated by Dodam *et al.* after intramuscular injection of acepromazine and oxymorphone, butorphanol and xylazine and diazepam and butorphanol versus a control group of canine patients[12]. This study showed that STT I values were significantly decreased 15-25 minutes following administration of medication where combinations of sedatives were used[12]. Medications were not evaluated in isolation and again, STT I was only measured for 25 minutes following administration. A study conducted by Ruiz *et al* in 2015 evaluated the effects intramuscularly administered tramadol had on STT I, IOP and PD values in dogs[42]. Prior to administration, STT I, IOP and PD were evaluated in all dogs and following this, tramadol was dosed at 4mg/kg or 6mg/kg, after which ocular parameters were only evaluated for a total of 60 minutes following intramuscular administration[42]. The researchers found that there was no statistically significant change in any of the ocular parameters evaluated; however, dogs only received one dose of the medication so inter-subject comparisons could not be made.[42]. Given the variability in how different patients metabolize tramadol, the lack of crossover in their study design may have prevented them from capturing significant effects[43,44].

A consistent, significant limitation of the afore-mentioned studies is that most of them evaluated STT for a limited period of time (minutes to 48 hours post medication administration maximum), and often STT I values were not evaluated more than twice in a single day. Most of these studies were also lacking in their approach to their statistics,

including justification of their sample size, consideration of an appropriate acclimation period, randomization, blinding and appropriate analysis of the data collected. Ultimately, what these studies have demonstrated is that we can expect a decrease from the apparent baseline tear production in dogs when they are administered sedatives and/or analgesics; however, we cannot establish long term trends from the data collected. We should also be mindful to interpret these results in light of the conditions in which the tests were performed, especially knowing the many potential pitfalls and limitations associated with performing STT I.

Some may consider the STT I to be a rudimentary and basic test when evaluated superficially; however, clearly, as with any other testing modality, the examiner must be mindful of appropriate testing technique, potential limitations and various external factors that have been shown to impact results. Despite the many documented limitations associated with the STT I, it remains the mainstay of evaluating quantitative tear production in veterinary medicine, and the gold standard for evaluating tear production in both a clinical and research setting. Ultimately, STT I should continue to be utilized as it is minimally invasive, inexpensive, readily available, fast, and relatively easy to perform, especially in the hands of an experienced operator. The small animal practitioner would do well to invest in learning how to appropriately perform this test, and critically interpret its results.

1.2.3 Intraocular Pressure and Rebound Tonometry

1.2.3.1 History and Methods of Testing

Intraocular pressure (IOP) is a result of the balance between the production of aqueous humor and its drainage by the iridocorneal angle and uveoscleral outflow[18]. Fluctuations in IOP negatively impact ocular health, as increases in IOP can threaten vision secondary to glaucoma and optic nerve degeneration, and decreases in IOP (hypotony) can result in cataract formation, corneal degeneration and retinal detachment[2]. Changes in IOP may be secondary to conditions causing ocular inflammation (uveitis), glaucoma, or external factors including medication administration such as propofol, alfaxalone and concurrent use of morphine, alfaxalone and midazolam[4,16,45,46]. Intraocular pressure is reported in mmHg, with a quoted normal value of 19 +/- 5.7mmHg for a canine patient (range 11-29mmgHg), depending on the tonometer used[47]. Intraocular pressure can be measured via manometry or tonometry. Manometry provides a direct measurement of IOP via paracentesis of the anterior chamber; however, due to its invasive and impractical nature, manometry is not utilized in veterinary clinical practice[48]. Tonometry, on the other hand is a non-invasive, but indirect estimation of the IOP, providing a measure of corneal tension, which is utilized to estimate IOP[2]. Currently, there are three recognized instrumental tonometry techniques in veterinary practice including indentation, applanation and rebound tonometry[2,48]. Digital tonometry, where the practitioner uses their fingers to exert pressure on the globe, thereby estimating the “hardness” of the eye has also been described; however, this technique provides crude, qualitative data only, and is no longer widely used[2].

The most commonly used indentation tonometer is the Schiotz tonometer, developed by Hjalmar August Schiotz in 1905. This tonometer evaluates the degree of corneal indentation, produced by a given weight when placed on the central cornea[2]. Due to limitations with its design and use in veterinary patients, this tonometer has been largely replaced by digital, handheld applanation and rebound tonometers. A commonly utilized applanation tonometer in veterinary medicine is the TonoPen®[b]. This applanation tonometer measures the force required to flatten an area of the cornea, thereby providing an estimate of IOP[48]. Disadvantages of the TonoPen® include the requirement for topical anesthesia to be applied to the cornea, the requirement for daily instrument calibration, variability in operator "force" when applying the tip of the tonometer to the cornea and subsequent inter-operator variability, a larger probe head versus the TonoVet®[c], and underestimation of true IOP values in conditions of increasing IOP[48]. Nevertheless, this tonometer is still widely used in small animal general practice as it is readily available, more user-friendly and accurate versus the Schiotz, and when used appropriately, provides a good estimation of canine IOP.

The latest developed method of evaluating indirect IOP is rebound tonometry. Rebound tonometry utilizes a small probe which contacts the cornea, and then rebounds to the instrument thereby measuring probe deceleration. Eyes with higher IOP will cause a more rapid probe deceleration resulting in a shorter time for the probe to return to the instrument[2]. Rebound tonometry is becoming widely utilized in veterinary medicine, with many ophthalmologists using the TonoVet® rebound tonometer in both clinical and research settings. The TonoVet® is considered easy to use, with practice, and is well

tolerated by veterinary patients. Unlike the TonoPen®, it does not require topical anesthesia or daily calibration, its small probe size allows specific areas of the corneal to be avoided, and it is readily utilized even in small patients[2,48,49]. Correct utilization requires that the probe be held at an angle of 90 degrees to the eye, 4-8mm from the surface of the cornea to ensure accuracy of measurements[48]. The instrument is very sensitive to any deviations in the angle at which it is held and must be calibrated/selected to the appropriate species; it also requires practice to become a proficient user.

1.2.3.2 Limitations and Available Research Studies

Although tonometry provides the veterinary practitioner with a clinically practical and clinically relevant method for evaluating IOP, there are several factors that should be considered when evaluating the accuracy and validity of IOP results even when utilizing a digital instrument. Intraocular pressure is influenced by several external factors including age and gender of the patient, patient handling, patient positioning, location of the probe on the cornea, the so-called “tonographic effect”, the type of tonometer used and the operator’s abilities, the time of day when a measurement is obtained, the need for an appropriately long acclimation period and effects of concurrent medications.

Intraocular pressure has been documented to change according to the age and gender of a canine patient[25]. A 2014 study conducted by Verboven *et al.* evaluated the IOP of eight healthy Beagles from the same litter, with both sexes being equally represented, between two and 12 weeks of age, on a weekly basis. Researchers found that IOP values increased significantly until six weeks of age, and again between 10 and 11

weeks[25]. Researchers also found that male dogs displayed a significantly higher IOP versus female dogs between 10 and 12 weeks of age; however, during this time period, male dogs were not housed in the same circumstances as their female counterparts[25] which may have led to errors due to a change in acclimation and degree of external stimulation. A quoted statistically significant decrease in IOP was noted between 11 and 12 weeks of age[25]. Although it is not routine to evaluate IOP in very young puppies, the examiner should note that IOP values may display variability as a patient ages, and initial values obtained may not represent that patient's true baseline.

Several studies have documented the effect of external pressure on IOP values, whereby pressure exerted on the neck or eyelids will cause an increase in IOP values[15,50,51]. A 2006 study performed by Pauli *et al.* evaluated the effect of neck leads in contrast to body harnesses on intraocular pressures of canine patients, and found that neck collars increased the IOP by a mean of 7.4mmHg within seconds of the pressure being exerted, versus a mean of 2.3mmHg noted with a body harness[50]. It is also well accepted that any pressure exerted on the globe itself will result in a potentially clinically relevant increase in IOP. A 2018 study demonstrated a significant increase in IOP in cats who underwent dorso-ventral extension of the eyelids[51]. A study performed in 2008 by Broadwater *et al.* evaluating the effect of body position on IOP in healthy canine patients demonstrated that there was "no significant change in IOP over time for dogs maintained in sternal recumbency, suggesting that this position may allow for the most consistent and repeatable IOPs"[15]. The study performed by Broadwater *et al.* found that the IOP value decreased in the 5-minute study period for seated dogs, but

was unchanged in sternally recumbent dogs[15]. Ultimately, these studies have demonstrated the requirement to provide limited, gentle restraint, and to maintain consistency in patient positioning to ensure IOP results are accurate and relevant.

Recent studies have also demonstrated the potential for variability in IOP results depending on the position of the tonometer on the cornea due to the variability in corneal thickness between the central and peripheral cornea. De Oliveira *et al.* found that deviation from the central cornea could result in over and under estimated values for IOP[14], emphasizing the importance of maintaining the tonometer probe at an angle perpendicular to the central cornea to ensure accuracy and reproducibility of results.

Another potential source of variability in IOP measurements between tonometers may be secondary to the tonographic effect where repeated attempts to obtain IOP using an applanation tonometer may artificially decrease IOP in canine patients[2,14]. The tonographic effects occurs secondary to tonography, which is a described technique where IOP is measured continuously typically for 2-4 minutes, by applying a tonometer to the corneal surface to allow for an estimation of conventional aqueous humour outflow[2]. This effect illustrates the importance of attempting to obtain as accurate a result as possible when evaluating a patient's IOP for the first time.

The type of tonometer used should always be documented and the same tonometer should be utilized for the same patient in both a clinical and research setting, due to variability in values obtained by different instruments[48]. A recent study by von Spiessen *et al.* comparing the TonoPen® (applanation tonometer) with the TonoVet® (rebound

tonometer), revealed that the TonoVet® consistently yielded higher IOP values in glaucomatous eyes versus the TonoPen®[48]. The authors in this study hypothesized that the TonoVet® may be better able to detect early stages of glaucoma[48]. This study also concluded that both tonometers evaluated were significantly influenced by the presence of corneal pathology and that the values obtained by the TonoPen® were significantly affected by the operator due to the need for manual pressure to be exerted on the cornea[48]. The authors emphasized the need to attempt to evaluate remaining healthy corneal tissue, whenever possible, for the most accurate determination of IOP[48]. Based on potential variability in measurements obtained between instruments, it is always recommended to note which tonometer was utilized to obtain any IOP values, so that results are as reproducible as possible for any subsequent examiner.

Intraocular pressure has been documented to be affected by circadian rhythm[16,33]. IOP has been documented to display a diurnal acrophase with "robust daily rhythms"[33], and a 2016 study performed by Meekins *et al.* documented that IOP values were significantly higher at 8AM versus at noon and 8PM in the dog[16]. This same study also revealed that "an acclimation period of at least 5 days is recommended…to minimize the influence of patient related factors such as stress on IOP measurements"[16]. The conclusion that an appropriate acclimation period is required resulted in a significant loss in sample size in the study performed by Meekins *et al.* This study serves as a reminder that an appropriate study design is critical to provide impactful and bias-free results. Given the diurnal variation in IOP and the fact that an acclimation period of at least five days is required to establish true baseline levels, evaluation of this ocular

parameter in a research setting must be appropriately and carefully planned, to capture natural variations in values and to avoid incorrect interpretation of results.

Several studies have also evaluated the effect of analgesics and sedatives on IOP values and their potential impact on ocular health and their effect on the success of ophthalmic procedures. The 2017 study performed by Mayordomo-Febrer *et al*. revealed a slight, but statistically significant increase in IOP in canine patients sedated with morphine, alfaxalone and midazolam, with the increase in IOP maintained following alfaxalone induction and sevoflurane anesthesia[4]. A 2016 study conducted by Doering *et al.* evaluated the effect of glycopyrrolate on IOP values and found that IOP was not affected by this anticholinergic medication[39]. A 2015 study performed by Kanda *et al.* documented a decrease in IOP secondary to administration of low and moderate doses of medetomidine and xylazine[40].

In veterinary medicine, estimation of IOP in canine patients continues to be measured via the use of different handheld tonometers. Clearly, every instrument for evaluating IOP in the dog carries its own set of potential pit-falls including operator error, changes secondary to robust circadian rhythms, challenges with handling and patient demeanor, and secondary to existing drug therapy. Investigators and clinicians alike would do well to remember the potential limitations of these measurement techniques and implement ways to mitigate their effects. Nevertheless, applanation and rebound tonometry remain the mainstay of estimating IOP values in canine patients in small animal practice.

1.2.4 Pupillary Diameter

1.2.4.1 History and Methods of Testing

Pupil size may be evaluated in a qualitative or quantitative manner[10,40]. In clinical practice, functionality of the iris is assessed by evaluating the pupillary light reflex (PLR) which is performed by shining a bright light into the ipsilateral pupil and appreciating a constriction (direct response), followed by a constriction of the contralateral pupil (consensual response). The PLR is a qualitative measure which provides information about the retina, cranial nerves II and III and the midbrain[2,52].

The purpose of the iris is to adjust the size of the pupil through the action of both dilator and constrictor muscles[2]. Dilation of the pupil is mediated by the sympathetic nervous system resulting in mydriasis, whereas constriction of the pupil is mediated by the parasympathetic nervous system resulting in miosis[2]. Amongst other functions, the adjustment in pupil size allows for regulation of the amount of light which may enter the posterior segment of the eye striking the retina, with an increase in light causing a constriction of the pupil[2], with normal quoted values of maximum 9.60+/-0.57mm and minimum 7.06+/-0.91mm[52]. Fluctuations in pupil size are possible secondary to a variety of physiologic and disease states which may result in changes to one or both pupils. Mydriasis may be seen in patients suffering from glaucoma, secondary to stress and in environments of lower ambient lighting. Conditions which result in uveitis may cause miosis secondary to ciliary muscle spasm as well as central nervous system disease (e.g. Horner's syndrome, cranial trauma) and secondary to environments with bright ambient lighting. The use of various medications may also impact pupillary

diameter and may result in detrimental or beneficial effects to the eye. For example, topical mydriatics including tropicamide and atropine will cause dilation of the pupil, whereas systemic propofol and etomidate have been shown to induce miosis in dogs (decrease of 1.18+/- 0.38mm for propofol, decrease of 4.44+/- 0.42mm for etomidate, p-value <0.01)[53]. Understanding the effect a medication has on pupil size is important due to its impact on IOP. A mydriatic pupil results in decreased aqueous humor outflow, and a subsequent increase in IOP, whereas a miotic pupil will increase outflow of aqueous humor with a subsequent decrease in IOP[2,40].

Visual assessment of pupil size is a qualitative test and extremely subjective, typically allowing the observer to appreciate significant changes in pupil size while subtleties are likely to be missed[54]. Quantitative pupil size may be measured via handheld pupillometer or via use of a handheld graduated ruler or calipers[10,52,53,55,56]. Use of handheld calipers provides a measurement of the PD in millimeters; however, this test is fraught with challenges in an awake individual as the examiner must read the value while the subject and their eye are able to move. Whiting *et al.* described a method for measuring pupillary diameter which involved continuous infrared illumination, combined with timed delivery of a visible light stimulus, and a camera for image capture[54]. Canine subjects were sedated and/or anesthetized for this procedure and stay sutures and a lid speculum was used to attempt to immobilize the eye. Despite the use of physical and chemical restraint, the researchers still observed apparently spontaneous fluctuations in pupil size[54], further illustrating the challenges associated with accurate evaluation of PD.

1.2.4.2 Limitations and Available Research Studies

There are few studies which have evaluated PD as a clinical outcome of medication administration, despite the potential impact changes in pupil size may have on ocular health and maintenance of aqueous humor equilibrium. A 2015 study by Kanda *et al.* found that there was no significant pupillary constriction following administration of the alpha-2 adrenergic agonists xylazine and medetomidine; however, the authors hypothesized that although no pupillary constriction was noted, mydriasis would have been inhibited due to the alpha-2 adrenergic action of both medications used[40]. Similarly, a 2015 study conducted by Kim *et al.* found that pupillary constriction velocity and maximum constriction velocity were decreased in patients who had received atropine, xylazine and ketamine[52]. This study also noted the potential utility of using a handheld pupillometer adopted from human medicine, to provide more accurate quantitative values for PD with recognized limitations due to the instrument's origins in human medicine[52]. In contrast, a 2017 pilot study conducted by Yeh *et al.* found that intravenous administration of dexmedetomidine resulted in frequent changes in pupil size[10]; subsequently, due to these fluctuations, the investigators administered additional sedative medications including acepromazine, propofol and isoflurane anesthesia to prevent changes in PD. Kovalcuka *et al.* found that IOP and horizontal pupillary diameter both increased with the use of ketamine and diazepam when used in healthy dogs, suggesting that these medications should be used with caution in patients suffering from glaucoma[55]. Similarly, a 2013 study performed by Gunderson *et al.* evaluated the effects of midazolam combined with either propofol and etomidate on

pupil size in normal dogs[53]. The authors found that both induction protocols resulted in a decrease in PD[53]. Whiting *et al.* found that despite the use of sedation including high dose dexmedetomidine, dexmedetomidine/ketamine, dexmedetomidine/butorphanol, the chemical restraint protocol that resulted in the least amount of spontaneous pupil size fluctuations was the use of dexmedetomidine and isoflurane anesthesia (2.7+/-1.3% versus 49+/-9.8% for dexmedetomidine high dose alone)[54]. The authors hypothesized that these spontaneous fluctuations in pupil size may be secondary to opposing input of the sympathetic and parasympathetic nervous systems, limiting the establishment of a consistent baseline pupillary diameter[54]. This finding further illustrates the challenges associated with accurately evaluating pupil size even with the aid of powerful sedative drugs. Consistent limitations of these studies include the available methodology of evaluating pupil size, and the lack of standardization and agreement between available methods of testing.

Unfortunately, there are few studies evaluating the effects of commonly used sedative and analgesic medications on pupillary diameter, and available studies have illustrated the challenges associated with measuring this ocular parameter. Even the use of chemical restraint and modified protocols to limit ocular movement have not necessarily mitigated the difficulties associated with collecting accurate data. This conclusion presents researchers and clinicians alike with an interesting conundrum, namely, whether the data we collect is truly indicative of the effect these medications have on pupil size or, does our testing methodology lack the ability to capture true biological changes.

1.2.5 Tear Break-Up Time

1.2.5.1 History and Methods of Testing

Tear break-up time (TBUT), or tear film break-up time, measures the time for the tear film to dissociate from the surface of the cornea and provides the examiner with an indirect measure of the integrity of the mucin and lipid portions of the tear film[57]. In human patients, TBUT is the test most commonly utilized to evaluate stability of the tear film in clinical practice[19].

In dogs, TBUT is performed by placing one drop of fluorescein stain into the eye and evaluating the surface of the cornea with a cobalt blue light filter until the first dry area (appreciated as a dark spot in the stained tear film) in the fluorescein stain is noted[19,57]. The eyelids are held open during this procedure, as timing to initial dry spot formation begins after the patient's last blink[22,24]. Normal values for TBUT in the dog have been cited as 21.53 +/- 7.42 seconds[22]. An accelerated TBUT (shorter time to observation of the first black spot) indicates a less stable tear film. Although clinically useful, this is a qualitative and extremely subjective test, with the potential for high variability in values obtained[22,57], with values being observer dependent. Tear break-up time has been observed to be decreased in canine diabetic patients[5] and secondary to administration of diphenhydramine[58].

1.2.5.2 Limitations and Available Research Studies

There is a dearth of available research evaluating the effects of medications on TBUT. Shepard *et al.* did evaluate TBUT as part of their 2011 study examining the effects of inhaled anesthetics on tear production in dogs; however, the main limitation of this study

was that TBUT was evaluated once a week only for the duration of the study[24]. Evans *et al.* found a statistically significant difference (p-value = 0.008) in TBUT values between day 1 of their study and day 21 (15.21+/- 6.38 seconds versus 8.08+/- 3.42 seconds) in dogs administered diphenhydramine; however, dogs were not evaluated on days 6-20 of the 21-day study period, so no results were available during the majority of the study[58]. Additionally, not all dogs with pre-existing ocular abnormalities were excluded from the study, including dogs with corneal abnormalities[58]. This study design is problematic due to the inclusion of dogs with pre-existing corneal disease, as the use of this medication may have had a greater impact on their lacrimation capabilities. A 2001 study performed by Saito and Kotani evaluated various methods of estimating lacrimation in healthy Beagles including TBUT[22]. The study by Saito and Kotani provided data to help establish baseline values for lacrimation in healthy dogs; however, there remains a significant gap in the literature evaluating the effects of systemically administered medications and their effects on lacrimation.

Ultimately, evaluating TBUT is challenging and prone to observer interpretation. Given the subjective nature of this test, results should be evaluated critically, and when necessary, repeated. Nevertheless, given the potential for medications to accelerate TBUT and destabilize the tear film, this test has its place in ophthalmologic-based pharmacological research studies.

1.2.6 Corneal Reflex and Corneal Touch Threshold

1.2.6.1 History and Methods of Testing

The corneal reflex is charged with protecting the cornea from damage by facilitating a blink and retracting the globe[2]. This reflex is extremely sensitive, and corneal esthesiometry provides a quantitative estimation of corneal sensitivity, called corneal touch threshold (CTT), which is an accepted estimation of corneal innervation[59–61].

Although there are various commercially available corneal esthesiometers, the Cochet-Bonnet esthesiometer[d] is most commonly utilized in veterinary medicine[2,62]. The Cochet-Bonnet esthesiometer utilizes a 0.12mm nylon filament, with a range in length of 0.5-6cm. Values in centimeters are then converted to g/mm^2 (range of 0.4-15.9g/mm^2) using a conversion table provided by the manufacturer[61]. To measure CTT, the end of the fully extended filament is touched to the central surface of the cornea until a slight bend is achieved in the filament. The corneal surface is touched several times in quick succession, and the length of filament which results in more than 50% of touches eliciting a blink reflex, is the CTT[61–64]. The shorter the filament, the more pressure is exerted on the cornea to elicit a blink reflex; therefore a less sensitive eye will have a lower CTT (a shorter filament)[63]. The different regions of the cornea have varying levels of sensitivity, with the central cornea being the most sensitive due to its richly innervated surface[2,64]. It is therefore critical to ensure the same area of the cornea is evaluated when measuring CTT. Observer timing is also important as the subject may spontaneously blink at the same time as the filament is stimulating the cornea. The test also carries with it an inherent risk of potential iatrogenic trauma to the corneal surface.

It is also important to note that the Cochet-Bonnet esthesiometer was developed for human patients, who have a much more sensitive cornea when compared to canine patients. It is therefore possible that the instrument is not calibrated appropriately to detect subtle changes in canine corneal sensitivity[63].

Factors that decrease corneal sensitivity will blunt the corneal reflex thereby increasing the potential for corneal trauma to occur. Corneal sensitivity has been documented to be decreased in brachycephalic breeds and is hypothesized to be an important factor in the development of corneal disease in these patients[65]. A documented decrease in corneal sensitivity has also been noted in patients with buphthalmos secondary to glaucoma, in those suffering from systemic illness, including diabetes mellitus and myasthenia gravis, and secondary to medication use including topical anesthetics, topical beta-blockers, topical prostaglandin analogues, topical carbonic anhydrase inhibitors and systemically administered diphenhydramine[2,58,60,61,64].

1.2.6.2 Limitations and Available Research Studies

As noted with PD and TBUT, there are very few research studies evaluating CTT. With respect to adverse effects with topical NSAIDs, Dorbandt *et al.* evaluated the effects of topical diclofenac and topical flurbiprofen and found that neither had an effect on CTT results when given up to twice daily for 30 days[63]. This study occurred in two phases. In phase I, the researchers evaluated CTT prior to administration of the drug, they then instilled five drops of the medication, and evaluated the corneal sensitivity every 15 minutes for a total of four measurements[63]. In phase II, CTT was performed prior to instillation of the drug, the patient was then treated at home for 30 days, and CTT was

evaluated following the 30-day period[63.] The authors observed that corneal sensitivity appeared to be decreased in conditions of higher humidity when compared to lower humidity levels, which may have confounded results[63]. The authors hypothesized that this apparent change in corneal sensitivity may have been secondary to the nylon filament absorbing more water, and thus becoming less rigid[63]. A study performed by Blocker *et al*. in 2007 evaluated changes in CTT in eyes with documented chronically high IOPs; however, this study did not evaluate effects of medical intervention on CTT results despite the instillation of prophylactic anti-glaucoma medications in non-surgery eyes[64].

Corneal touch threshold remains a gold standard test in the evaluation of corneal sensitivity in the dog. As with most other testing modalities, consistency in performing the test is paramount to ensuring accuracy of results. It is also critical to control environmental parameters as much as possible, to mitigate potential changes in the rigidity of the filament, or, consider use of a new filament whenever testing is performed. The test should also be approached with respect and care, as it may cause avoidable, iatrogenic trauma to the cornea, and unnecessary pain to the canine subject.

1.3 Medication Overview

1.3.1 Introduction

Treatment of acute and chronic pain in canine patients is common practice in veterinary medicine. Commonly prescribed oral analgesic medications used both alone, and in combination, include gabapentin, tramadol and meloxicam. These drugs are all readily available, typically well tolerated, considered largely effective,are relatively inexpensive and may be administered concurrently to a canine patient. Patients are typically treated with these medications for several days at a time, and yet, despite their widespread use, very little is known about their effects on the canine eye.

1.3.2 Gabapentin

1.3.2.1 Pharmacology

Gabapentin is commonly prescribed in veterinary medicine for its anticonvulsant and analgesic effects in canine patients. It is a neuropathic pain analgesic, and a structural analog of gamma aminobutyric acid (GABA), which appears to be most adept at treating chronic pain and neurologically-based pain in companion animals[1,66]. Gabapentin modulates the influx of calcium by binding calcium channels[66]; however, the exact mechanism of its analgesic action is not completely understood[1,66]. Oral doses of 50mg/kg have resulted in 80% bioavailability at two hours post-administration in canine patients with the drug being primarily excreted via the kidneys[1]. The drug has a quoted terminal half-life of 3.3 hours when dosed at 10mg/kg and 3.4 hours when dosed at 20mg/kg[67]. Evaluation of the drug in healthy Greyhounds by KuKanich and Cohen confirmed that 10-20mg/kg every 8 hours would maintain a plasma concentration of

2ug/kg, which is considered effective in humans, although effective concentrations in the dog are unknown[67]. Oral doses in dogs for pain control are cited as 10-20mg/kg every 8-12 hours. The most common reported adverse effects are sedation and ataxia[1].

1.3.2.2 Gabapentin and Ocular Research

Evaluation of gabapentin's effects on ocular parameters in canine patients is limited. A 2017 study performed by Trbolova *et al.* evaluated the effect of gabapentin on IOP in canine patients, when used as a premedication prior to induction of anesthesia with propofol[68]. In this two-arm parallel study, dogs were given compounded gabapentin at 50mg/kg by mouth, two hours prior to induction, or a placebo gel capsule. They were then induced using propofol, intubated and anesthesia was maintained using propofol[68]. Intraocular pressure was measured using the TonoPen® immediately prior to induction, immediately following induction, and five, ten and 15 minutes following induction by the same investigator blinded to treatment assignment. Interestingly, the researchers found that in the treatment group, there was no increase in IOP values as compared to the control group. They ultimately suggested that premedication with gabapentin may be useful for dogs undergoing ophthalmic procedures, where preventing a spike in intraocular pressure may be beneficial[68]. It should be noted that the dogs in the afore-mentioned study did not undergo an acclimation period to establish proper baseline IOP values, nor were any other ocular parameters evaluated. A 2017 study performed by Anfuso *et al.* demonstrated that application of topical 0.5% gabapentin effectively reduced both clinical signs and biomarkers of uveitis in rabbits *in vivo* and *in vitro* following injection of LPS (lipopolysaccharide)[66]. The researchers in this study ultimately

concluded that further investigation of gabapentin's utility in treatment of uveitis should be pursued[66]. It should be noted that no justified sample size was included for this study, nor did the researchers explain why they elected to evaluate aqueous humor samples for inflammatory mediators at seven and 24 hours post LPS injection. Excitingly, a 2019 study performed on human patients suffering from dry eye disease and concurrent neuropathic ocular pain, revealed an improvement in STT I and TBUT results (p-value <0.001) in those patients treated with gabapentin in addition to the standard treatment of topical lacrimometics and artificial tears versus control over a six week period[69]. This same study also noted an increase in comfort level in those patients treated with gabapentin versus control[69]. Self-admittedly, the researchers noted a limited sample size, and that only those patients with severe dry eye disease were included[69]; nevertheless, it would be interesting to see whether a similar improvement would be seen in canine patients.

Based on available literature, it appears that gabapentin may prove to be a useful adjunctive medication in the treatment of ocular disease. Touted as an effective neuropathic pain analgesic, this conclusion is perhaps not surprising given the close association and relationship between the eye and central nervous system. Additional research into whether gabapentin may prove beneficial for canine patients with ocular disease, whether as a topical or systemic formulation, should be considered.

1.3.3 Tramadol

1.3.3.1 Pharmacology

Tramadol is a “synthetic *mu*-receptor opiate-like agonist” with the ability to inhibit the reuptake of both serotonin and norepinephrine[1,70]. Both the opioid and non-opioid actions of tramadol contribute to the drug’s analgesic effects[1,44]. Following oral administration, the drug is metabolized via several metabolic pathways, and is recognized to have significant interpatient variability with regards to bioavailability following oral absorption[1,43,44,71]. The drug displays a complex metabolism and bioavailability and clearance are variable with quoted terminal half-lives of 1.1-2.2 hours[43,44]. Oral doses in dogs are variable and exhibit a wide range, with quoted doses ranging between 2-10mg/kg, typically given every eight hours[1,72]. Commonly observed side effects are related to the drug’s effect on the central nervous system, including sedation, agitation and tremoring[1]. Gastrointestinal side effects have also been observed including changes in stool consistency (constipation to diarrhea), vomiting and inappetence[1].

1.3.3.2 Tramadol and Ocular Research

There is a paucity in the literature evaluating the effect of tramadol on ocular parameters in dogs. A 2011 pilot study by Clark *et al.* examined how effective oral tramadol was at treating ocular pain when compared to topical nalbuphine or placebo[11]. In this study, dogs were randomly assigned to one of three treatment groups, following which, the researchers created a 4mm long corneal wound in the right eye of every dog. Dogs were then treated with topical nalbuphine (1 drop every 8 hours), oral tramadol

(dosed at 4mg/kg every 8 hours) or topical saline (1 drop every 8 hours) and all dogs received gentamicin topically[11]. To evaluate the effectiveness of therapy between treatment groups, the researchers utilized a pain scoring system to evaluate the dogs' comfort levels and instituted rescue therapy as required based on their individual scores[11]. Although the researchers noted that fewer dogs in the tramadol group required rescue therapy versus the other groups, these results were not statistically significant, likely secondary to an inadequate sample size. As previously noted, a study conducted by Ruiz *et al* in 2015 evaluated the effects intramuscularly administered tramadol had on STT I, IOP and PD values in dogs[42]. Prior to administration, STT I, IOP and PD were evaluated in all dogs and following this, tramadol was dosed at 4mg/kg or 6mg/kg intramuscularly[42]. The researchers found that there was no statistically significant change in any of the ocular parameters evaluated; however, dogs only received one dose of the medication so inter-subject comparisons could not be made[42]. Given the variability in how different patients metabolize tramadol, the lack of crossover in their study design may have prevented them from capturing significant effects[43,44]. Similarly, Santos *et al.* found no change in STT I or PD values in their 2013 crossover study when tramadol was administered at a dose of 2mg/kg intramuscularly[73]; however, they only evaluated STT I and PD a total of three times following administration until 45 minutes post-administration, canine subjects were not randomized to treatments, nor were the investigators blinded to treatment assignments[73].

Existing studies evaluating tramadol and its ability to positively impact the eye have been disappointing. Thus far, research has not supported that tramadol would provide

appreciable analgesia for the eye and the available literature is fraught with limitations that make drawing conclusions regarding its effects on ocular parameters evaluated challenging.

1.3.4 Meloxicam

1.3.4.1 Pharmacology

Meloxicam is a COX-2 preferential non-steroidal anti-inflammatory drug (NSAID)[1]. It is commonly prescribed in veterinary medicine for its analgesic, anti-pyretic and anti-inflammatory properties and may be given intravenously, subcutaneously or orally. Oral formulations of the drug are well absorbed, with peak plasma levels documented at 7-8 hours following administration, and a quoted average half-life of 24 hours in the dog[74]. The drug is primarily metabolized in the liver, and eliminated through urine and feces[1,74]. Canine oral doses for meloxicam include a one-time loading dose of 0.2mg/kg, followed by 0.1mg/kg maintenance doses every 24 hours (as per the manufacturer's recommendations[e]). Meloxicam is available both as a generic formulation and under the trade-marked name Metacam™, which has been available in Canada since 1998 for the treatment of pain secondary inflammation in in canine patients[75]. Reported adverse effects include vomiting, diarrhea, melena, and anorexia. Less common side effects include nephrotoxicity and hepatotoxicity as well as inhibition of platelet function[1,74].

1.3.4.2 Non-Steroidal Anti-Inflammatory Medications and Ocular Research

Non-steroidal anti-inflammatory medications are considered the most commonly prescribed class of systemic analgesic prescribed in veterinary medicine[76]. Given their widespread use, it is considered likely that a canine patient may be treated with an

NSAID at least once in their lifetime, and critically evaluating potential adverse effects of NSAIDs in canine patients is tantamount. Interestingly, the 2013 systematic review of available prospective studies in the veterinary literature performed by Monteiro-Steagall *et al.*, found that few studies were "appropriately designed to determine the safety of NSAIDs"[76]. The same review did not note any reported ocular adverse effects associated with systemic NSAID use in the articles reviewed.

The number of studies evaluating the effect meloxicam and other NSAIDs have on ocular parameters in dogs appear to be few. A study undertaken by Ribeiro *et al.* in 2009 aimed to evaluate whether meloxicam, when given to dogs by three different routes (subcutaneously, subconjunctivally, and topically) could inhibit ocular prostaglandin E_2 (PGE_2) production and subsequent protein influx into the anterior chamber[77]. Their results suggested that meloxicam, regardless of route of administration, did not inhibit PGE_2 synthesis[77]. Gilmour *et al.* compared the effects of systemically administered placebo (sodium chloride), meloxicam, carprofen and flunixin meglumine on PGE_2 concentrations in aqueous humor samples following aqueocentesis in healthy dogs[78]. Their results revealed that of the NSAIDs evaluated, and compared to placebo, flunixin meglumine significantly reduced the concentration of PGE_2. The researchers concluded that this NSAID may be an appropriate treatment choice to reduce intraocular inflammation in canine patients undergoing surgery ($p\text{-value} < 0.05$)[78]. Although the researchers in the afore-mentioned study showed a statistically significant difference between treatment groups, samples from six eyes were lost, overall sample size was not justified, dogs were not randomly assigned to treatment

groups, and the investigators were not blinded to treatment assignment[78]. Komnenou *et al.* evaluated carprofen in combination with medetomidine, propofol and halothane in their 2013 study. These authors found that dogs treated with this protocol had a significant decrease in their STT I values until two hours post-operatively. The main limitation of this study includes the fact that the drugs were not evaluated in isolation and STT I values were not measured beyond two hours[27]. A 2011 study performed by Pinard *et al.* evaluating the effects of oral carprofen vs placebo revealed no significant change in IOP between groups of healthy Beagles following anterior chamber paracentesis (p-value = 0.859)[79]. However, it should be noted that the dogs in this study did not undergo an acclimation period, nor was the sample size for the study justified[79]. Meekins *et al.* also evaluated the effects of oral carprofen versus placebo gel on IOP in healthy dogs, and found that twice daily dosing of carprofen had no statistically significant effect on IOP (p-value not provided)[16]. This study identified that an appropriate acclimation period is required for IOP values to return to baseline following data analysis[16]. These study design weaknesses may have falsely impacted their results, and the study would have benefited from a sounder design and statistical methodology.

1.4 Summary

1.4.1 Justification

This literature review has demonstrated the need to further evaluate the effects commonly administered systemic analgesics may have on ocular health. Furthermore, this review has identified that significant gaps exist in available research in terms of providing a complete overview of ophthalmic health and the effects of commonly administered medications on the canine eye. There can be no doubt that further research is warranted given the potential for pharmacologic interventions to cause damage to the canine eye. In addition, beneficial effects of commonly administered analgesics should be further elucidated.

1.4.2 Research Objectives

The objective of this research project is to evaluate the effects of repeated orally administered gabapentin, tramadol, meloxicam on ocular parameters in healthy dogs when given for three days.

1.5 Footnotes

a. Eagle Vision, Katena Products Inc, Denville, New Jersey, USA
b. TonoPen®, Reichert Inc, Depew, New York, USA
c. TonoVet®, ICare Finland Oy, Ayritie 22, Fi-01510 Vantaa, Finland
d. Cochet-Bonnet esthesiometer, Luneau Ophthalmologie, Chartres, France
e. Metacam package insert/product information, Metacam, Boehringer-Ingelheim, Burlington, Ontario, Canada

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2 Chapter 2: Effects of oral gabapentin, tramadol and meloxicam on ocular parameters in healthy dogs

2.1 Abstract

Objective

To determine the effects of oral gabapentin, tramadol and meloxicam on Schirmer tear test (STT), intraocular pressure (IOP), pupillary diameter (PD), tear break-up time (TBUT) and corneal touch threshold (CTT) when given to healthy dogs for three days.

Animals

Nine, healthy, intact male Beagles

Procedures

A randomized, blinded, case-crossover study using a 6-sequence, 3-treatment, 3-period design was performed. The study design included a 7-day acclimation period, three, 3-day treatment periods each followed by a 7-day washout period. Dogs received gabapentin (10mg/kg PO q8hr), tramadol (3mg/kg PO q8hr), or meloxicam (0.2mg/kg PO once, then 0.1mg/kg PO q24). Schirmer tear test, IOP and PD were performed four times daily. Tear break-up time was performed once daily and CTT was performed once during the acclimation period and each treatment and washout period. Measurements from days 1-5 and 6-7 of the acclimation period were compared and analyzed for statistical significance (p-value < 0.05). A generalized mixed effects linear regression

model was created for each parameter, accounting for repeat measures within individuals.

Results

Intraocular pressure was the only significantly different parameter in the last two days of the acclimation period (p-value 0.0021). Gabapentin and tramadol significantly affected only IOP when accounting for phase, day, time of day, animal ID, baseline value, and eye (-0.45 and -0.35mmHg, respectively; p-value 0.038).

Conclusions and Clinical Relevance

Our findings confirm that a minimum 5-day acclimation period is necessary for canine IOP measurements to return to baseline. Although gabapentin and tramadol did decrease IOP, this effect is not considered clinically significant.

2.2 Introduction

Canine patients are prescribed a variety of analgesics including neuropathic pain analgesics, opioids and non-steroidal anti-inflammatory drugs (NSAIDs) for treatment of acute and chronic painful conditions and diseases. These medications may be used alone or in combination and are often prescribed for several days. Although systemic side effects of these medications have been well researched in veterinary medicine, ocular side effects have not been well documented. Analgesics that decrease tear production, alter intraocular pressure, alter pupil size, expedite tear break-up time, or decrease corneal sensitivity may predispose a canine patient to development of ocular

pathology, or worsen existing ocular disease[1]. This potential impact is increased in patients with breed predispositions (e.g. brachycephalic breeds) and those suffering from systemic diseases including renal failure, heart failure, endocrinopathies and gastrointestinal disease[2–4]. Medications that impair tear production or destabilize tear film integrity, may predispose a patient to developing ulcerative keratitis. Similarly, medications that increase IOP may worsen existing glaucoma, or act as a catalyst in a patient predisposed to development of glaucoma. Medications that reduce corneal sensitivity may result in a stunted blink reflex, thereby increasing a patient's risk of developing corneal ulceration.

To provide a true overview of the effect a medication may have on ocular health, a comprehensive ophthalmic evaluation should be considered, including the effect a medication has on tear production, intraocular pressure, pupillary diameter, tear film stability and corneal sensitivity. This information would enable veterinary practitioners to prevent potentially harmful ocular sequelae and mitigate the occurrence of adverse drug reactions. Unfortunately, there are significant gaps in the literature regarding existing knowledge of the effects of common analgesics on ocular health. Available research studies have focused on a single analgesic, or systemically administered sedatives and induction agents[3,6–10], with most studies focusing on one or two parameters of ocular health and typically for short time periods[3,6–11]. Despite the frequency with which they are prescribed, the authors were unable to find any studies evaluating the effects of oral gabapentin, tramadol and meloxicam on ocular health in dogs.

Given the existing gaps in the current veterinary literature regarding the effects of commonly prescribed oral analgesics on ocular parameters in dogs, and the potential for systemic analgesics to affect the canine eye both detrimentally and beneficially, a more complete and long-term analysis of the impact of analgesics on ocular health is justified. The objective of this research study was to evaluate the effects of oral gabapentin, tramadol and meloxicam on ocular parameters in healthy dogs when given for several days. Based on available literature, the authors hypothesized that gabapentin would decrease intraocular pressure and corneal sensitivity, but have no effect on tear production, pupil size or tear film stability. Further, that tramadol would decrease corneal sensitivity, but have no effect on tear production, intraocular pressure, pupil size and tear film stability. Lastly, that meloxicam would have no effect on tear production, intraocular pressure, pupil size, tear film stability or corneal sensitivity.

2.3 Materials and Methods

Approval for the following study was obtained from the University of Guelph Animal Care Committee (Animal Utilization Protocol #3955) and all procedures were performed in accordance with the Canadian Council on Animal Care's guidelines.

2.3.1 Animals

Nine intact male, purpose-bred Beagles (CanCog, formerly Vivocore Inc., Toronto, Ontario) were used in this study and were housed at the Central Animal Facility (CAF, Guelph, Ontario) for the duration of the study. Sample size was calculated using the equation to determine a difference in means[12] based on the variable IOP. Based on the 2017 study conducted by Mayordomo-Febrer *et al.*, values of $\alpha = 0.05$, $\beta = 0.80$, a

standard deviation of 3.0mmHg[3] and an expected difference in means of 2.5mmHg resulted in the need for eight dogs. To account for any animal that may drop out of the study due to illness or injury, an additional dog was added. All Beagles were 10 months of age and underwent a GPE, and had comprehensive bloodwork, including a CBC and biochemical profile performed[a]; a baseline weight was also recorded for every Beagle. A complete ophthalmic examination was performed on both eyes of every dog prior to entry to the study including: a neuro-ophthalmic exam (dazzle reflex, menace response, palpebral reflex, pupillary light reflexes), Schirmer tear test[b], rebound tonometry[c], fluorescein stain[d], slit-lamp biomicroscopy[e], indirect ophthalmoscopy and fundic examination following administration of 1% tropicamide[f]. All GPEs were performed by the same investigator (AKS) and all ophthalmic examinations were performed by the same investigator (CLP) on the first day of the acclimation period.

All canine subjects underwent an acclimation period of seven days. The dogs were housed in the same room, and in the same groups (dogs 1 and 2, dogs 3 and 4, dogs 5 and 6, dogs 7, 8 and 9) for the duration of the study. The Beagles were encouraged to socialize in small groups when not being evaluated and environmental enrichment was provided to all dogs throughout the study (beds, toys, blankets, bones). The Beagles were fed an appropriate ration, determined by their body weight, of a commercially available canine kibble once daily[g], and had free access to fresh water at all times.

During the acclimation period, the Beagles were acclimatized to handling, ocular procedures, and administration of wet food at set times (8AM, 4PM, 12AM) to mimic medication administration during the treatment period (Table 1). The dogs were

exposed to 15 hours of light (5:30AM to 8:30PM) and nine hours of darkness (8:30PM to 5:30AM) over a 24-hour period, with a brief period (15-20 minutes) of light exposure necessary to accommodate feeding of wet food at midnight. Dogs were walked by CAF handlers immediately after the 4PM evaluation time only during the acclimation period and immediately after the 2PM evaluation time during the treatment and washout phases. The dogs also underwent a daily GPE at 2PM, including evaluation of temperature, pulse and respiration, and were evaluated daily to ensure they were urinating and defecating regularly and eating with a good appetite. Fecal consistency and gross urine colour were evaluated on a daily basis. The dogs were re-weighed the day before every treatment phase to ensure accuracy of medication dosing. A recheck CBC and biochemical profile were evaluated on the last day of both washout periods.

2.3.2 Study Protocol and Procedures

A randomized, blinded, case crossover study was used using a 6-sequence, 3-treatment, 3-period design. The study design consisted of three, 3-day treatment periods, with a seven-day washout period in between treatment periods (Figure 1). The dogs were randomly assigned by the statistician to one of three groups for every treatment period using block randomization (six possible sequences - ACB, ABC, BAC, BCA, CBA, CAB) to ensure all phases were balanced with three animals on each of the drugs for each phase of the study. Poker chips for the three drugs (3As, 3Bs and 3Cs) were placed in a bag, the statistician withdrew a chip for each dog and this determined the starting drug for phase I. The same procedure occurred for phase II after the initial

drug had been removed. Phase III drug was chosen by default to complete the sequence (Table 2).

Treatment groups consisted of gabapentin (Drug A; 100mg/mL; OVC Pharmacy, Ontario Veterinary College, University of Guelph, Guelph, Ontario, Canada) dosed at 10mg/kg every 8 hours[13], tramadol (Drug B; 10mg/mL; OVC Pharmacy, Ontario Veterinary College, University of Guelph, Guelph, Ontario, Canada) dosed at 3mg/kg every 8 hours[13] and meloxicam (Drug C; 1.5mg/mL; Metacam, Boehringer-Ingelheim, Burlington, Ontario, Canada) dosed at a loading dose of 0.2mg/kg on the first day, followed by a maintenance dose of 0.1mg/kg every 24 hours for the remaining 2 days (as per the manufacturer's recommendations; Metacam package insert/product information) (Figure 2). Gabapentin and tramadol were administered at 8AM, 4PM and 12AM to mimic owner administration at home. Meloxicam was administered once daily at 8AM. Those dogs receiving meloxicam were administered an equal volume of water via syringe at 4PM and 12AM to ensure the technician administering the medications remained blinded to medication assignment. All medications were administered by the same technician (BLF) in a meatball of wet food[h], or via syringe. The primary investigator (AKS), was not present for any treatments, and was blinded to all dogs' treatment assignments for the duration of the study. The Beagles were separated for all medication administration and watched to ensure the medication was consumed. Wherever possible, the medication was administered in wet food in individual, 6'' diameter metal dishes, which were evaluated to ensure all food and medication had

been consumed. When necessary, medication was syringe fed into individual subjects' mouths.

The Beagles were always evaluated in the same order, beginning with dog 1 and ending with dog 9. Data collection occurred in the same windowless room in the CAF, in close proximity to the kennel location[14]. The room door was closed during every evaluation, to limit noise and light contamination. Room temperature and humidity were recorded at the start of every ophthalmic evaluation for every dog[i]. Light was recorded at every measurement time[j], with the investigator seated with the subject in front of them. The light meter was permanently mounted at the approximate eye level height of the canine subject, facing the investigator.

During the seven-day acclimation period, dogs were acclimated to handling, and ocular measurement techniques. The initial five days were deemed necessary to ensure IOPs had returned to baseline[15]. The remaining two days of the acclimation period provided baseline values for all intraocular measurements. The Beagles were evaluated four times per day at 6:30AM, 11AM, 2PM and 7PM, in the 48 hours prior to the first treatment phase, during the three days of every treatment phase, and on the first washout day of each washout phase (Tables 1 and 3). On days 2-7 of washout, the Beagles were evaluated at 6:30AM, 2PM and 7PM every day (Table 4). At each time slot, STT, IOP and PD were evaluated. All measurements were performed in the same sequence, at each evaluation time, with STT being performed first, followed by rebound tonometry, then PD. Tear break-up time when performed, was evaluated last. Tear break-up time was evaluated at 6:30AM, and CTT (when performed) was evaluated at

7PM (Table 4). Evaluation times were selected to ensure changes in IOP and tear production secondary to diurnal rhythm, were captured[6,15,16], and to evaluate STT, IOP and PD three, and six hours post-medication administration. All ophthalmic evaluations were performed by the same investigator (AKS), and restraint of the canine subjects was performed by the same technician (BLF) with the exception of a single day in the last washout period where a different restrainer was required (CAB). The dogs were placed in a seated position on a raised examination table using minimal restraint. The dogs' heads were held in a natural position[15,17,18], with the technician placing one hand under the mandible, and the other hand at the back of the cranium to maintain head position at a 90 degree angle to the examination room table. No digital pressure was exerted around the eye, orbit or neck, thereby limiting any potential variability in IOP secondary to changes in body position or digital pressure[19,20]. All evaluations were carried out on the left eye (OS) followed by the right eye (OD).

Schirmer tear test was performed by inserting the notched end of one sterile STT strip into the central, ventral conjunctival sac and held there for 60 seconds, with values recorded in mm/min[21]. Rebound tonometry was performed using the same TonoVet® tonometer, with a new probe[k] utilized for each Beagle per evaluation time, per the manufacturer's recommendation[17]. Intraocular pressure was measured with the TonoVet® probe perpendicular to the central cornea[18,18,22], with the instrument appropriately calibrated for a canine patient. The first, error free IOP reading was taken as correct and recorded[18,22]. Schirmer tear test strips, TonoVet® probes and fluorescein strips from the same production batch, with identical lot numbers, were utilized

throughout the duration of the study to prevent possible inconsistencies[14,16,23,24]. Pupillary diameter was recorded in millimeters (mm) as the horizontal pupil size using handheld ophthalmic calipers[25,26,l] by evaluating first the left eye, resetting the calipers, and then evaluating the right eye. Tear break-up time was performed every morning at 6:30AM by applying a concentrated drop of fluorescein to each eye, blinking the eyelids once, and measuring the time (in seconds) until development of dark spots/lines appeared in the fluorescein stained tear film of the dorso-lateral cornea, using an ophthalmoscope with a cobalt blue light filter[1,m]. The fluorescein strip was moistened with sterile saline[n] and a drop applied OS to determine TBUT. The strip was re-moistened and a drop applied OD and TBUT was determined. Residual fluorescein stain was flushed from both eyes using sterile saline[n] after TBUT was performed. Corneal touch threshold was evaluated using a Cochet-Bonnet esthesiometer containing a 0.12mm nylon filament[o], during the acclimation phase, on day three of every treatment phase and on day four of both complete washout phases. A new nylon filament was utilized for the study and the esthesiometer was stored per the manufacturer's recommendations for the duration of the study. Corneal touch threshold was performed with the nylon filament extended to its maximum length of six centimeters (cm) and advanced perpendicularly to touch the central cornea, until a slight bend was achieved in the filament. The filament was shortened by 0.5cm increments, until three out of five touches elicited a blink reflex, determining the CTT[1,27], reported in units of cm. Immediately following the CTT evaluation, and 12 hours afterwards, the dogs' eyes were evaluated for evidence of corneal erosions or ulcers, by

instilling fluorescein stain into both eyes by gently touching a moistened fluorescein strip to each dorsal bulbar conjunctiva, and evaluating for corneal defects with the use of a cobalt blue light filter[1].

2.3.3 Statistical Analysis

All data was initially recorded by an assistant using pen and paper at the time that ocular measurements were performed. Following this, two technicians independently transcribed all the collected hard-copy data into separate Excel spreadsheets. Spreadsheets were then compared for any discrepancies using a conditional formatting rule in Excel. Wherever a discrepancy was encountered, the hard-copy data was consulted prior to making any corrections in Excel. Data was cleaned manually and then a master data sheet was created. All descriptive statistics, hypothesis testing and linear regression were estimated using the same statistical computing software[p].

2.3.3.1 Descriptive Statistics

All continuous variables were checked for normality using a combination of the Shapiro-Wilk normality test and visual assessment of a histogram prior to calculating summary statistics. When a variable was normal the mean was calculated, where the data was non-parametric the median was calculated (Table 5). Baseline mean values for each parameter were calculated from the measurements collected during the acclimation period. The mean/median from the first five days of acclimation was compared to the final two days of acclimation to determine if there was any significant change in the measure that occurred as the dogs grew accustomed to the facility, investigators and study protocols, using a paired t-test or Wilcoxon signed rank test and cut-off p-value of

0.05. Univariable comparisons were made for each of the ocular measurements by drug and dog using the Kruskal Wallis test for variables which were not normally distributed, and a one-way ANOVA for normally distributed variables where visual evaluation of a boxplot confirmed that variances were equal (Table 6).

2.3.3.2 Linear Regression

A linear mixed model was constructed for each ocular measure (STT, IOP, PD, TBUT and CTT), therefore five models in total were created. The dependent variable was the ocular measure during the study (excluding the acclimation period). The fixed effects were mean baseline ocular measure, phase (I-III), day (1-10 for phases I and II and days 1-5 for phase III), time of day (7AM, 11AM, 2PM, 7PM), eye (OS, OD), drug (A, B, C), temperature (°C), humidity (%) and light (number of luxes). Baseline ocular measures were based on the mean or median value of each parameter during the acclimation phase. Various random effect and repeat measures combinations were investigated as well as correlation structures. Each model was constructed using backwards elimination. All two-way and three-way interactions were included. The model that converged and had the lowest Akaike Information Criterion (AIC) was chosen. The parameter estimates were based on restricted maximum likelihood estimation (REML) and residuals were examined for normality and homoscedasticity visually through plotting of residuals and using normality tests such as the Shapiro-Wilk test and transformations of the dependent variable were transformed (i.e. log), and subsequently back-transformed, if needed.

2.4 Results

All nine dogs completed the acclimation period and all three treatment and washout phases (32 days in total) (Table 5 & Table 7). One canine subject displayed a single episode of vomiting immediately following medication administration (tramadol - Drug B). Medication administration was not repeated, and the subject's vomiting resolved spontaneously. The models were each run with and without the one data point after the vomiting episode (2PM measures) and no change was noted in the parameter estimates or variable significance in any of the models, therefore the value was left in. A single canine subject (dog 8) developed superficial corneal abrasions following CTT (OS on both occasions), detected on routine fluorescein staining with no other signs of ocular disease on two separate occasions (initial occurrence on the first day of the second washout period, which resolved spontaneously within eight hours and without treatment; second occurrence on the last treatment day of the last treatment phase which resolved spontaneously within 11 hours and without treatment). Healing of corneal abrasions was documented by serial fluorescein staining at every evaluation time, following initial documentation, until the subject stained fluorescein negative. A single canine subject developed a superficial corneal laceration following CTT (dog 5 OD on day five of the first washout period) which was treated with corneal debridement using a sterile, cotton tipped applicator and administration of a topical antibiotic[q] and an ocular lubricant[r] during the washout period only. The corneal laceration was documented to be fluorescein stain uptake negative 30 hours after treatment was instituted, and treatment was discontinued at this time.

Baseline CBC and biochemistry profile results were all unremarkable. Five dogs displayed a mild elevation in band neutrophils on repeat CBCs performed on the last day of the first washout period and eight of nine canine subjects displayed a mild elevation in their urea values on repeat biochemical profiles performed on the last day of the first washout period. Repeat CBCs performed on the last day of the second washout period were unremarkable. Repeat biochemical profiles performed on the last day of the second washout period revealed mild elevations in urea in six of the nine canine subjects. Normal creatinine values were noted for all canine subjects at every evaluation time.

During the study, the dogs were re-weighed three times, with a weight range of 9.0-13.2kg over the course of the study. No statistically significant weight change was noted for any of the dogs during any phase of the study (p value = 0.609) (Figure 3).

Temperature in the room utilized to collect all ocular data was measured between 22-23 degrees Celsius and humidity of 47-70% for the duration of the study. Light level in the room utilized to collect all ocular data was measured between 148-190 luxes for the duration of the study (Table 8).

2.4.1 Statistical Analysis Acclimation Period

Ocular measurements collected on the first five days of the acclimation period (A) were compared to the last two days of the acclimation period (B) to evaluate for statistically significant differences between the two periods. Results from the acclimation period did not reveal any statistically significant difference between values collected between days

1-5 versus days 6-7, with the exception of IOP (Table 9). Since IOP was significantly different in the last two days of the acclimation period, this data was designated as baseline and used to determine the mean or median baseline values for all parameters in the study. This baseline measure was then used as a fixed effect in the linear models.

2.4.2 Statistical Analysis Treatment Period

2.4.2.1 STT

The mean STT during the acclimation period was 21.1mm/min (95% CI: 20.8-21.4mm/min). The mean values between the first five days (21.2mm/min) and the last two days (21.1mm/min) were not significantly different (p-value = 0.7563). The overall mean STT value for all dogs during the three phases of the study was 21.0mm/min (95% CI: 18.2-23.9mm/min). The mean STT values for the nine dogs while receiving each of the three drugs was: Drug A (gabapentin) 20.9mm/min (95% CI: 17.9-23.8mm/min), Drug B (tramadol) 21.1mm/min (95% CI: 18.1-24.1mm/min), Drug C (meloxicam) 21.1mm/min (95% CI: 18.2-21.1mm/min) (Figure 4). These values were not significantly different using the Kruskal Wallis test (p-value = 0.7330). The STT results did differ over the day that the measures were collected (F = 2.595; p-value = 0.05). Based on the Tukey post hoc test, the difference occurred between the hours of 630AM and 5PM (when STT was 0.5mm/min higher in the evening than the morning) (p-value = 0.05) (Figure 5). Using a linear regression model with a random effect for ID*Drug*Phase*Eye there were only three significant fixed effects in the final model for STT and an interaction between “Day” and “Time of Day” (therefore day has to remain

in the model despite having a p-value greater than 0.05) (Table 10). Drug did not have a significant effect on the STT measure (p-value = 0.8602).

2.4.2.2 IOP

The mean IOP during the acclimation period was 14.5mmHg (95% CI: 14.2-14.8mmHg). The mean value between the first five days (14.8mmHg) and the last two days (14.1mmHg) was significantly different (p-value = 0.0021). The mean IOP value for all dogs during the three phases of the study was 14.0 mmHg (95% CI: 13.9-14.1mmHg) while the median was 14.0mmHg. The mean IOP values for the nine dogs while receiving each of the 3 drugs was: Drug A 13.8mmHg (95% CI: 11.6-16.1mmHg), Drug B 13.8mmHg (95% CI: 11.6-16.0mmHg), Drug C 14.1mmHg (95% CI: 11.8-14.1mmHg) (Figure 6). These values were considered significantly different using the Kruskal Wallis test (p-value = 0.0185). The IOP results also differed over the period of the day that the measures were collected (F = 24.25; p-value < 0.0001). Based on the Tukey post hoc test the difference occurred between the hours of 630AM and all other time points (IOP was ~1.0mmHg higher at 630) (p-value < 0.0001) (Figure 7). Peak IOP values (14.3mmHg) occurred on Day 4 of a phase and was significantly higher than all other days except Day 1 (14.0mmHg) and 2 (14.0mmHg). The IOP was also significantly higher in the phase III of the study (14.0mmHg) in comparison to phase I (13.9mmHg) and II (13.9mmHg). Using a linear regression model and repeat measures for Drug*Day*ID*Per*Eye with a toep2 correlation structure there were six significant fixed effects in the final model for IOP and two interactions (Table 11). Drug had a significant effect on the IOP measure (p-value = 0.0379) with Drug A and B having a

lower mean ocular pressure than C and baseline. The interaction terms baseline*drug and baseline*time of day means that the effect of drug and time of day varies by the dog's baseline level to begin with (i.e. dogs with lower baseline values at the start were less affected by Drug C and time of day, 630AM, than those with higher baseline values). The IOP was also significantly different between eyes with the right eye (evaluated second) having a 0.5mmHg lower pressure than the left eye (evaluated first) on average.

2.4.2.3 PD

The mean PD during the acclimation period was 9.8mm (95% CI: 9.6-9.9mm). The mean values between the first five days (9.82mm) and the last two days (9.72mm) were not significantly different (p-value = 0.4325). The mean PD value for all dogs during the three phases of the study was 8.8mm (95% CI: 8.7-8.8mm) while the median was 9.0mm. The mean PD values for the nine dogs while receiving each of the three drugs was: Drug A 8.6mm (95% CI: 7.8-9.4mm), Drug B 8.6mm (95% CI: 7.8-9.3mm), Drug C 8.5mm (95% CI: 7.8-9.2mm) (Figure 8). These values were not significantly different from one another using the Kruskal Wallis test (p-value = 0.2704). The PD results also differed over the period of the day that the measures were collected (F = 26.17; p-value < 0.0001). Based on the Tukey post hoc test the difference occurred between the hours of 630AM and all other time points (PD was 0.2-0.36mm lower at 630) (p-value < 0.0001) (Figure 9). A linear regression model with a random effect for Drug*Day*ID*Phase was constructed and there were six significant fixed effects in the final model for PD and 2 interaction terms (Table 12). Drug did not have a significant

effect on the PD measure (p-value = 0.1877); however, baseline PD was significantly higher than the PD measured throughout the three phases of the study (p-value = 0.0224). The variable for eye yielded a p-value of exactly 1.0 which indicates there was no variation between the PD in the left and right eye.

2.4.2.4 TBUT

The mean TBUT during the acclimation period was 17.6sec (95% CI: 13.4-21.7). The mean value between the first five days (17.37sec) and the last two days (18.02sec) was not significantly different (p-value = 0.4325). The mean TBUT value for all dogs during the three phases of the study was 17.4sec (95% CI: 13.7-21.2sec) while the median was 17.3sec. The mean TBUT values for the nine dogs while receiving each of the three drugs was: Drug A 17.2sec (95% CI: 13.4-20.9sec), Drug B 17.6sec (95% CI: 14.0-21.2sec), Drug C 17.5sec (95% CI: 13.8-21.1sec) (Figure 10). These values were not significantly different using the Kruskal Wallis test (p-value = 0.5720). As the study progressed through phases I-III TBUT significantly increased in phase III (mean = 18.6sec) in comparison to phase I (mean = 16.9sec; p-value < 0.0001) and phase II (mean = 17.3sec; p = 0.0120). Using a linear regression model that accounts for random effects between Phase*ID*Drug the only significant fixed effects in the model were phase (p = 0.0480) and day (p = 0.003). Drug did not have a significant effect on the TBUT (p = 0.7291). Notably this model and the CTT model were the only ocular parameters not different from baseline.

2.4.2.5 CTT

The mean CTT during the acclimation period was 3.9cm (95% CI: 2.9-4.8cm). CTT was evaluated once during the acclimation period only, so a comparison between the first five days and the last two days could not be performed. The mean CTT value for all dogs during the three phases of the study was 2.8cm (95% CI: 1.6-3.9cm) while the median was 2.5cm. The mean CTT values for the nine dogs while receiving each of the three drugs was: Drug A 2.6cm (95% CI: 1.6-3.6cm), Drug B 2.6cm (95% CI: 1.5-3.8cm), Drug C 2.4cm (95% CI: 1.5-3.3cm). These values were not significantly different using the Kruskal Wallis test (p-value = 0.6253). Using a linear regression model that accounts for random effects in Drug*Phase*ID*Day there was only one significant fixed effect in the final model for CTT (Day; p-value < 0.0001). The CTT measured on day three was 0.3s shorter than day seven. Drug did not have a significant effect on the CTT measure (p-value = 0.3196) (Figure 11) nor did baseline (p-value = 0.0567).

Some significant non-associations to note are the lack of effect that carry-over had on the models (p-values all > 0.05) and lack of effect of the environmental parameters (temperature, humidity and light) on all ocular measures except for the effect of light on PD (p-value = 0.0008).

2.5 Discussion

The objective of this study was to evaluate whether the analgesics gabapentin, tramadol and meloxicam have beneficial or adverse effects on healthy canine eyes, when given orally over three days. The study protocol was designed to capture a large volume of information regarding the drugs' effects on five different ocular parameters, selected

due to their clinical relevance on the ocular health of canine patients. This resulted in the collection of a very large, and complex data set (n = 1890 for STT, IOP and PD), which allowed for a more complete and appropriate statistical analysis of the effects these analgesics have on the ocular parameters evaluated. The breadth and scope of the current study's data set ultimately enabled the authors to examine the data for biological interactions and effects that other studies have been unable to address in addition to the impact that the drugs may have had. To the authors' knowledge, this study presents one of the most comprehensive ocular studies conducted to date on canine subjects. Available studies evaluating the effect of sedatives, analgesics and general anesthesia on ocular parameters have evaluated only one or two measures of ophthalmic testing, and have typically performed those evaluations over a 24-48 hour time period only[3,6–11,26]. These other study protocols resulted in the collection of far fewer data points. For example, the 2017 study performed by Mayordomo-Febrer *et al.* resulted in a total of 88 data points for STT and 88 data points for IOP[3]; similarly, the 2015 study performed by Jang *et al.* resulted in the collection of 120 data points for IOP[6] (both of the afore-mentioned studies utilized the TonoPen® to measure IOP values). When compared to the current study where 1890 data points were collected for STT, IOP and PD, it is possible that the smaller data sets may have prevented the investigators from establishing enough statistical power to appreciate nuances in statistical and biological trends.

The acclimation period was evaluated as two separate periods due to results published by Meekins *et al* in their 2016 study which showed that canine IOP values took five days

to return to baseline levels[15]. Although STT, PD, TBUT and CTT did not reveal a statistically significant difference between acclimation period A versus B, our results did reveal a difference in IOP between period A and period B and support the conclusion that a minimum of five days is required for IOP values to return to baseline in healthy dogs[15]. Similarly, the washout period of seven days was adequate, ensuring the preceding drug had no appreciable impact on the ocular parameters in subsequent phases. Clearly, an appropriately long acclimation period is necessary to help ensure collected data is as representative of a patient's true clinical state as possible. Potential reasons for changes in ocular parameters may include a canine subject's stress level at the time of testing, patient handler abilities, operator technique, and challenges associated with instrumentation[19,22,28–30]. For example, a handler may require time to establish appropriate restraint techniques to limit any pressure around the eye or orbit. Similarly, an operator may require time to become a proficient user of the TonoVet® as the instrument is very sensitive to any deviations in the angle at which it is held.

Tear production, evaluated via STT, was not significantly different during the study between the three drugs. While the linear model indicates that STT is statistically different from the baseline STT the effect is not clinically significant (20.9mm/min vs. 21.1mm/min). A statistically significant effect was also noted for time of day, which was mainly driven by an increasing STT as the day progressed (Figure 5). These results are consistent with previously documented findings that have shown a nocturnal peak in tear production[16,31]; however, the authors could find no available veterinary-based research studies evaluating the effect of gabapentin or meloxicam on STT I results in

canine patients, nor could they find any studies investigating the impact of independent variables such as time of day, phase and baseline values on STT I.

Intraocular pressure was significantly higher in phase III of the study (14.0mmHg) in comparison to phase I (13.9mmHg) and II (13.9mmHg). With respect to intraocular pressure, this study revealed that of the three drugs evaluated, only gabapentin and tramadol had a statistically significant effect on IOP, with a resultant decrease of 0.45mmHg and 0.35mmHg, respectively. This decrease in IOP is not considered clinically significant in healthy animals, but this effect should be further evaluated in animals exhibiting clinical disease associated with glaucoma. The variation in ocular pressure between the left (11.6) and right eye (12.1) was unexpected but could be explained by the lack of randomization between eyes. During the study the left eye was always examined first. This finding serves as a reminder to randomize all aspects of clinical trials, including eyes. Intraocular pressure was noted to be highest at 630AM and appeared to decrease as the day went on. This finding is consistent with previously published reports that IOP displays a diurnal acrophase[15,16]. Available studies that have evaluated IOP after administration of oral gabapentin and intramuscular tramadol did not allow for inter-subject comparisons as no crossover design was used[32], and evaluated IOP for 15 minutes following administration only[33]. The authors could find no studies evaluating the effect of meloxicam on IOP in dogs.

Pupillary diameter was significantly higher in the acclimation period than at any time during the study. This finding is consistent with an anticipated increase in stress and subsequent sympathetic tone during the acclimation period resulting in mydriasis[1,34].

This finding suggests that pupillary diameter should be evaluated following an appropriate acclimation period to ensure accuracy of results and that our acclimation period of seven days may not have been long enough to attain true baseline levels. Another interesting finding was the lack of variation in PD between eyes, while this is expected based on the method by which measurements were obtained[35], it was surprising that there was zero variation between eyes (p-value = 1.0). Light has a significant impact on PD, however it is a challenge to tease out the relationship due to the interaction terms. A closer look at the data demonstrates a large variability in the light levels measured at 10AM and 7PM (luxes varied between 148-190) which is likely the reason that PD is impacted. All PD measurements on all subjects were obtained in the same, windowless room, which was located inside another windowless room with zero external light contamination. The reason for the variability in light levels may be due to fluctuations in the actual lighting systems and/or neon lightbulbs utilized by the CAF. Again, the authors could find no studies evaluating the effects of gabapentin or meloxicam on PD in canine patients.

For the two tests TBUT and CTT, there was a reduced number of datapoints due to the less frequent number of tests run (n = 576 for TBUT and n = 108 for CTT). With less data it becomes a challenge to detect any changes among the variables even if one was present (i.e. lack of power). Phase had a significant impact on TBUT. With each subsequent phase the TBUT increased (I-II + 0.366sec, II-III + 1.337sec, I-III + 1.704sec). The reason for this increase is unknown; however, may be secondary to an increase in operator proficiency when performing this subjective test. The CTT test

results were only impacted by day, with baseline just over the borderline for significance determination ($p = 0.0567$). It appears that the dogs tolerated the corneal touching for a significantly longer length of filament on day 7 (0.3cm longer) than on day 3. This finding is unrelated to phase so is challenging to simply attribute to a learned tolerance of the procedure.

Few adverse effects were noted during the present study. The first adverse effect was related to performing CTT. As CTT involves touching the corneal surface with a nylon wire, iatrogenic corneal trauma secondary to CTT evaluation can occur. The second adverse event was related to a single episode of vomiting in one canine patient following administration of tramadol. This episode of vomiting may have been secondary to syringe feeding of the medication, or stress associated with handling. Since there was no impact on the models upon removal of this datapoint, it is felt this vomiting episode had little impact on the results.

Anomalies noted on bloodwork may be considered an adverse event; however, at no time did any canine patient display clinical signs secondary to the abnormalities noted. Abnormalities noted on the results of the CBCs were attributed to delays in sample preparation[36] and were considered spurious following blood smear evaluation by a veterinary clinical pathologist[s]. The mild elevations in urea may have been secondary to a delay in sample separation, hemolysis or a non-fasted sample[36] and were not considered clinically significant as no canine patient displayed any signs of systemic disease and creatinine values were all within normal limits[s]. Similarly, the subsequent mild elevations in urea noted on repeat biochemical profiles were not considered

clinically significant as no signs of systemic disease were present. Additional testing including a urinalysis to rule out pre-renal/renal causes of elevated urea could have been pursued; however, this was not considered necessary as creatinine values were always within normal limits, and no signs of systemic disease were noted for any canine subject. Additional testing to rule out gastrointestinal disease resulting in elevated urea (eg. fecal analysis including evaluation for occult blood) was not pursued as no canine subject any signs of systemic disease including grossly abnormal stool, nor were all subjects with elevated urea receiving meloxicam concurrently.

Limitations of the present study include lack of diversity in the study population, as only young, intact male Beagles were utilized, and results may not be representative of the effect of these medications in the general canine population. The use of liquid compounded gabapentin and tramadol was determined to be necessary for the current study, to ensure the dose of medication administered to each canine subject was as accurate as possible. These medications were compounded by a veterinary pharmacy, using strict quality control practices and verified compounding practices[37,38,t]; however, inaccuracies in the quoted concentration of medications administered is possible. Future studies that use compounded medications may consider verifying quoted doses of the administered medications utilized. The current study used only a single dose for gabapentin and tramadol; it is possible that at higher doses statistically and clinically significant effects on the ocular parameters evaluated may be appreciated. In the future, comparison of different dose ranges for the evaluated medications may be informative. As previously stated, there was a single day where a different restrainer (CAB) was

used; however, this change in restrainer was not noted to have any effect on the data collected. It would also have been advisable to randomize which eye was evaluated first.

Our study revealed that gabapentin, tramadol and meloxicam, when given at the quoted doses for three consecutive days, should have no clinically adverse effects on tear production, intraocular pressure, pupillary diameter, tear film stability or corneal sensitivity in healthy dogs. The present study did not reveal a decrease in corneal sensitivity, suggesting that these analgesics do not provide a clinically significant analgesic effect on the cornea. Based on the results of this study, and the statistically significant impact eye had on STT and IOP values, we suggest that future studies randomize which eye is evaluated first, to mitigate potential interactions of this effect. This finding is in contrast to other studies which did not find a statistically significant difference between eyes[15,16]. The present study design ensured the collection of over 1800 units of data (STT, IOP, PD) allowing for the detection of even the smallest difference in summary statistics, enabling a comprehensive statistical analysis to be performed. Another unique feature of our study is secondary to the complex mixed linear regression models created, allowing us to account for the dependency of repeat measures within the same dog, at multiple times, days and phases. Not accounting for this dependency violates the assumptions of independence for many routine statistical tests, therefore mixed models should be used in these situations. All the final models converged and had homoscedasticity of the residuals therefore we are confident in their structure and results.

This study sought to address the existing knowledge gap of the effect that oral analgesics have on ocular parameters in healthy dogs. Future studies could include evaluation of various dose ranges of commonly prescribed oral analgesics, their effects on a more representative study population, and examination of their effects in patients with pre-existing ocular diseases.

2.6 Footnotes

a. Animal Health Laboratory, Laboratory Services Division, University of Guelph, Ontario, Canada
b. Eagle Vision, Katena Products Inc, Denville, New Jersey, USA
c. TonoVet® Type TVO1, Tiolat Oy, Finland
d. Fluorescein sodium, Optitech Eyecare, Med Devices Lifesciences Limited, London UK
e. Kowa SL-15 portable slit-lamp, Kowa, Tokyo, Japan
f. Mydriacyl 1%, Alcon Canada Inc., Mississauga, Ontario, Canada
g. Purina Pro Plan Savor Adult Chicken and Rice Formula, Purina Canada, Mississauga, Ontario, Canada
h. Royal Canin Gastrointestinal High Energy Canine, Guelph, Ontario, Canada
i. Acurite Indoor Thermometer and Humidity Monitor, Lake Geneva, Wisconsin, USA
j. Fisher Scientific, Fisherbrand Traceable Dual-Range Light Meter, Ottawa, Ontario, Canada
k. TonoVet® probes ICare Finland Oy, Ayritie 22, Fi-01510 Vantaa, Finland
l. Storz Jameson Caliper E-2410, Bausch & Lomb Incorporated, Heidelberg, Germany
m. Welch Allyn Ophthalmoscope, Skaneateles Falls New York, USA
n. Bausch and Lomb Sensitive Eyes Saline Plus, Bausch & Lomb, Rochester, New York, USA
o. Cochet-Bonnet esthesiometer, Luneau Ophthalmologie, Chartres, France
p. R version 5.3.1/R Studio version 1.1.456 R Foundation for Statistical Computing, Vienna, Austria
q. Tobramycin 0.3%, Sandoz Canada, Mississauga, Ontario, Canada
r. Tear-Gel®, Bausch & Lomb Incorporated, Valeant Canada, Laval, Quebec, Canada
s. Personal communication Dr. Kristiina Ruotsalo DVM, DVSc, DACVP, clinical pathologist and adjunct faculty, Animal Health Laboratory, University of Guelph, Guelph, Ontario, Canada

t. Personal communication, Heather Kidston RPh, Ontario Veterinary College Health Sciences Centre Pharmacy Manager, Ontario Veterinary College, Guelph, Ontario, Canada

2.7 Tables

Table 1. Acclimation period measurement protocol

	Day 1, 2, 3, 4	**Day 5**	**Day 6, 7**
6:30	STT, IOP, PD, TBUT *CBC, biochemistry (*Day 1 only) Meatball	STT, IOP, PD, TBUT Meatball	STT, IOP, PD, TBUT Meatball
11:00	/	/	STT, IOP, PD
14:00	STT, IOP PD, TPR	STT, IOP PD, TPR	STT, IOP, PD, TPR, Weigh (Day 7)
16:00	Walk Meatball	Walk Meatball	Walk Meatball
19:00	STT, IOP, PD	STT, IOP, PD, CTT, fluorescein stain	STT, IOP, PD
24:00	Meatball	Meatball	Meatball

Table 2. Randomized, blinded, 6-sequence, 3-treatment, 3-period case crossover study protocol

ID	AP	Phase I	WO I	Phase II	WO II	Phase III
		Drug		Drug		Drug
Dog 1	7d	C	7d	A	7d	B
Dog 2	7d	A	7d	B	7d	C
Dog 3	7d	B	7d	C	7d	A
Dog 4	7d	A	7d	C	7d	B
Dog 5	7d	C	7d	B	7d	A
Dog 6	7d	B	7d	A	7d	C
Dog 7	7d	B	7d	C	7d	A
Dog 8	7d	C	7d	A	7d	B
Dog 9	7d	A	7d	B	7d	C

AP = acclimation period

Drug A = gabapentin

Drug B = tramadol

Drug C = meloxicam

WO = washout period

Table 3. Treatment period measurement protocol

	Day 1	**Day 2**	**Day 3**	**Day 4**
6:30	STT, IOP, PD, TBUT	STT, IOP, PD, TBUT	STT, IOP, PD, TBUT	STT, IOP, PD, TBUT
8:00	Oral gabapentin Oral tramadol Oral meloxicam	Oral gabapentin Oral tramadol Oral meloxicam	Oral gabapentin Oral tramadol Oral meloxicam	Meatball
11:00	STT, IOP, PD	STT, IOP, PD	STT, IOP, PD	STT, IOP, PD
14:00	STT, IOP, PD, TPR	STT, IOP, PD, TPR	STT, IOP, PD, TPR	STT, IOP, PD, TPR
16:00	Oral gabapentin Oral tramadol Oral saline	Oral gabapentin Oral tramadol Oral saline	Oral gabapentin Oral tramadol Oral saline	Meatball
17:00	Walk	Walk	Walk	Walk
19:00	STT, IOP, PD	STT, IOP, PD	STT, IOP, PD, CTT, fluorescein stain	STT, IOP, PD
24:00	Oral gabapentin Oral tramadol Oral saline	Oral gabapentin Oral tramadol Oral saline	Oral gabapentin Oral tramadol Oral saline	Meatball

Table 4. Washout period measurement protocol

	Day 1	**Day 2, 3, 5, 6**	**Day 4**	**Day 7**
6:30	STT, IOP, PD, TBUT Meatball	STT, IOP, PD, TBUT Meatball	STT, IOP, PD, TBUT Meatball	STT, IOP, PD, TBUT CBC, Biochemistry Meatball
11:00	STT, IOP, PD	/	/	/
14:00	STT, IOP, PD, TPR	STT, IOP, PD, TPR	STT, IOP, PD, TPR	STT, IOP, PD, TPR, Weigh
16:00	Walk Meatball	Walk Meatball	Walk Meatball	Walk Meatball
19:00	STT, IOP, PD	STT, IOP, PD	STT, IOP, PD, CTT, fluorescein stain	STT, IOP, PD
24:00	Meatball	Meatball	Meatball	Meatball

Table 5. Descriptive Statistics of nine Beagles who participated in a 3-period, 6-sequence crossover study evaluating the impact of gabapentin, tramadol, meloxicam on ocular parameters

Variable	Range	Mean (95% CI)	Median	Test of Normality
Weight	9.0-13.2	11.0 (9.8-12.2)	10.8	p = 0.02724
STT (N = 1890)	11.0-31.0	21.0 (18.2-23.9)	21.0	p < 0.0001
IOP (N = 1890)	8-23	14 (13.9-14.1)	14	p < 0.0001
PD (N = 1890)	6.0-13.0	8.8 (8.7-8.8)	9.0	p < 0.0001
TBUT (N = 576)	**6.2-27.7**	**17.4 (13.7-21.2)**	**17.3**	**p = 0.6539**
CTT (N = 108)	1.0-6.0	2.8 (1.6-3.9)	2.5	p < 0.0001

CI = 95% confidence interval

Table 6. Ocular measures of nine Beagles during three drug 6-sequence crossover study

	Drug				
Variable	AP (95% CI)	A (95% CI)	B (95% CI)	C (95% CI)	p-value
STT (N = 1890)	21.1 (18.5-21.1)	20.9 (17.9-23.8)	21.1 (18.1-24.1)	21.1 (18.2-21.1)	p = 0.7330
IOP* (N = 1890)	**14.5 (11.9-17.1)**	**13.8 (11.6-16.1)**	**13.8 (11.6-16.0)**	**14.1 (11.8-14.1)**	**p = 0.0185**
PD (N = 1890)	9.8 (8.6-10.9)	8.6 (7.8-9.4)	8.6 (7.8-9.3)	8.5 (7.8-9.2)	p= 0.2704
TBUT[§] (N = 576)	17.6 (13.4-21.7)	17.2 (13.4-20.9)	17.6 (14.0-21.2)	17.5 (13.8-21.1)	p = 0.5720
CTT	3.9 (2.9-4.8)	2.6 (1.6-3.6)	2.6 (1.5-3.8)	2.4 (1.5-3.3)	p = 0.6253

[§]Kruskal-Wallis test performed except for variable TBUT (ANOVA test)

*significantly different $p < 0.05$

Table 7. Comparison of ocular measures by dog during 6-sequence crossover study

	ID								
Variable	1	2	3	4	5	6	7	8	9
STT*	19.6	21.7	21.7	**24.3**	19.8	19.4	21.0	20.1	21.6
IOP	14.0	12.1	13.9	13.5	14.6	15.8	12.1	**16.4**	13.5
PD	8.7	8.7	8.7	**8.4**	8.7	8.8	**9.0**	8.6	8.7
TBUT	16.8	17.4	16.8	17.3	17.2	17.0	17.2	**19.2**	**18.2**
CTT	2.6	2.5	**3.7**	3.2	2.5	2.3	**3.0**	2.8	2.4

Table 8. Measurements of environmental parameters over 32-day crossover study of ocular measures in nine Beagles

Variable	Range	Mean (95% CI)	Median	Test of Normality
Temperature (N =1686)	21.0-23.0	22.0 (21.7-22.4)	22.00	$p < 0.0001$
Humidity (N = 1695)	46.0-70.0	60.2 (55.0-65.4)	61.00	$p < 0.0001$
Light (N = 1890)	148.0-191.0	177.2 (169.4-185.0)	179.0	$p < 0.0001$

Table 9. Comparison of ocular measurements of both eyes from nine Beagles between acclimation period A and acclimation period B prior to entering a 3-treatment, 3-washout period crossover study

Variable	A (Days 1-5)	B (Days 6-7)	p-value
STT (N = 324)	21.2 (20.8-21.5)	21.1 (20.6-21.6)	0.7563
IOP (N = 324)	**14.8 (14.4-15.2)**	**14.1 (13.8-14.5)**	**0.0021**
PD (N = 324)	9.82 (9.6-10.0)	9.72 (9.6-9.9)	0.4325
TBUT (N = 126)	17.4 (13.3-21.5)	18.0 (13.6-22.4)	0.4325
CTT (N = 18)	3.9 (2.9-4.8)	NA	NA

Table 10. Final linear regression mixed model for Schirmer tear test results of nine Beagles participating in a 3-phase crossover clinical trial evaluating gabapentin, tramadol and meloxicam

Fixed effects	p-value
Drug	0.8602
Day	0.1102
Time of day	0.0020*
Baseline STT	0.0474*
Baseline STT^2	0.0389*
Day*Time of day	0.0321*

*p-value < 0.05

Table 11. Final linear regression mixed model for intra-ocular pressure results of nine Beagles participating in a 3-phase crossover clinical trial evaluating gabapentin, tramadol and meloxicam

Fixed effects	p-value
Drug	0.0379
Phase	0.0010
Eye	< 0.0001
Day	< 0.0001
Time of Day	< 0.0001
Baseline IOP	< 0.0001
Base IOP*Time of Day	< 0.0001
Base IOP*Drug	0.0046

Table 12. Final linear regression mixed model for pupillary diameter results of nine Beagles participating in a 3-phase crossover clinical trial evaluating gabapentin, tramadol, and meloxicam)

Fixed effects	p-value
Drug	0.1877
Day	< 0.0001
Time of Day	0.0001
Baseline PD	0.0224
Base PD *Base PD	0.0214
Light	0.0008
Light * Light	0.0008
Light * Time of Day	0.0002

2.8 Figures

Figure 1. Research Project Timeline

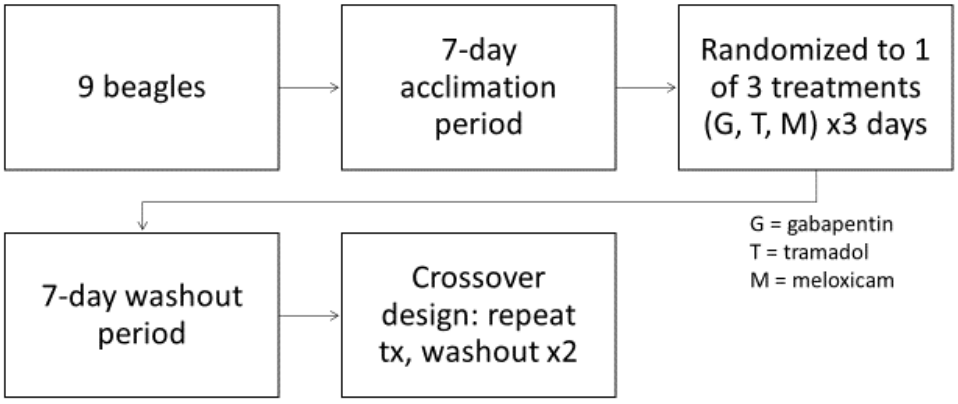


Figure 2. Research Project Design

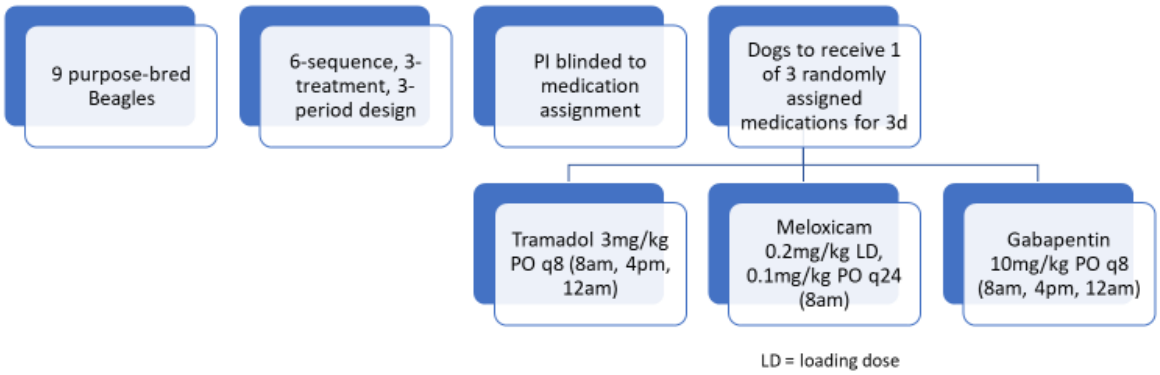


Figure 3. Boxplot of weight of nine Beagles according to phase during 6-sequence crossover study

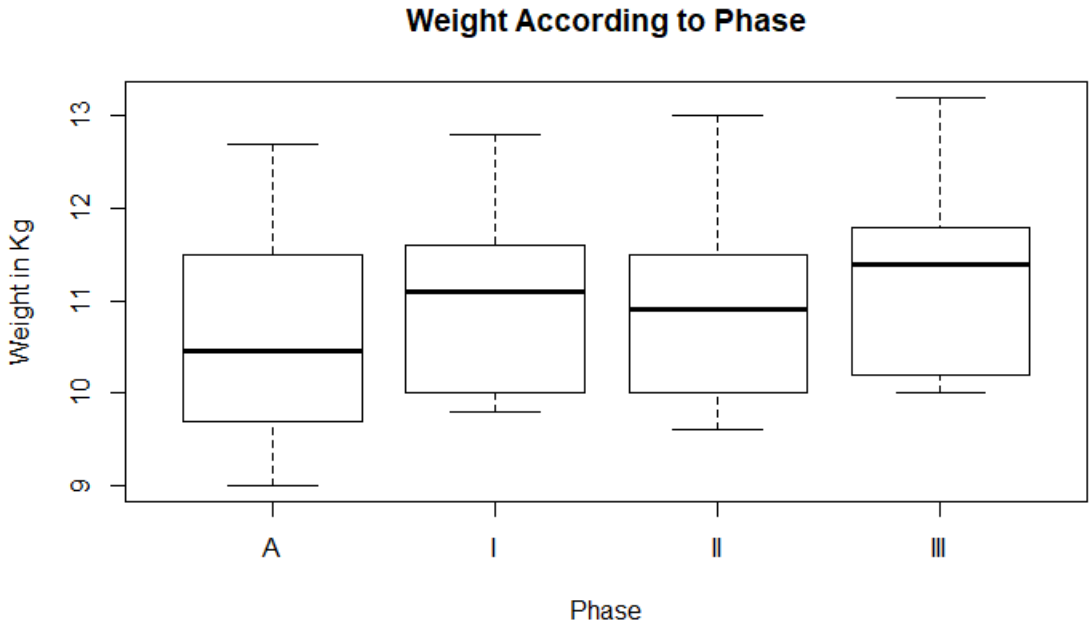


Figure 4. Schirmer tear test results of nine Beagles administered three oral analgesics (gabapentin, tramadol or meloxicam) for the first three days (dashed line) of a 10-day phase in comparison to the baseline value from the acclimation period (solid line)

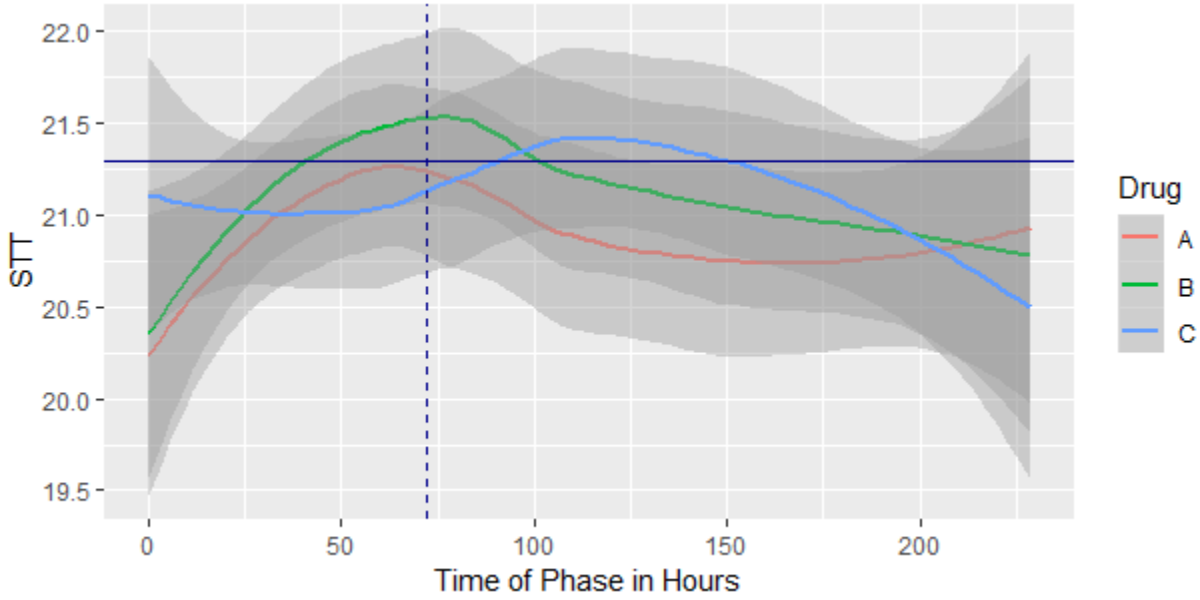


Figure 5. Schirmer tear test results of nine Beagles administered three oral analgesics (gabapentin, tramadol or meloxicam) over a 24-hour period in comparison to baseline

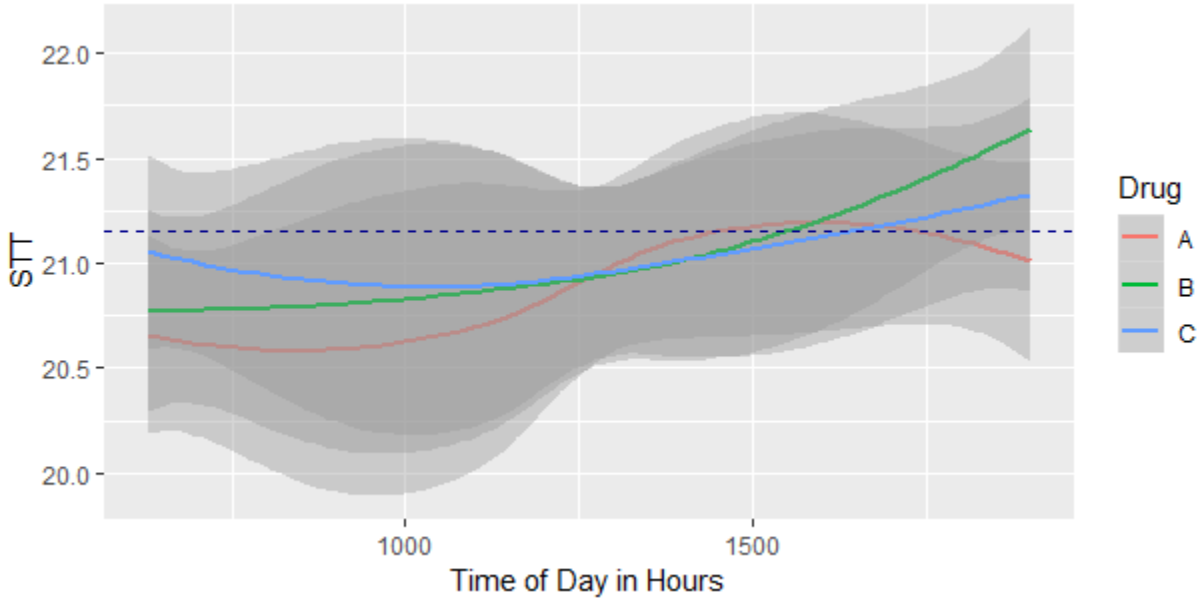


Figure 6. Intra-ocular pressure test results of nine Beagles administered three oral analgesics (gabapentin, tramadol or meloxicam) for the first three days (dashed line) of a 10-day phase in comparison to the baseline value from the acclimation period (solid line)

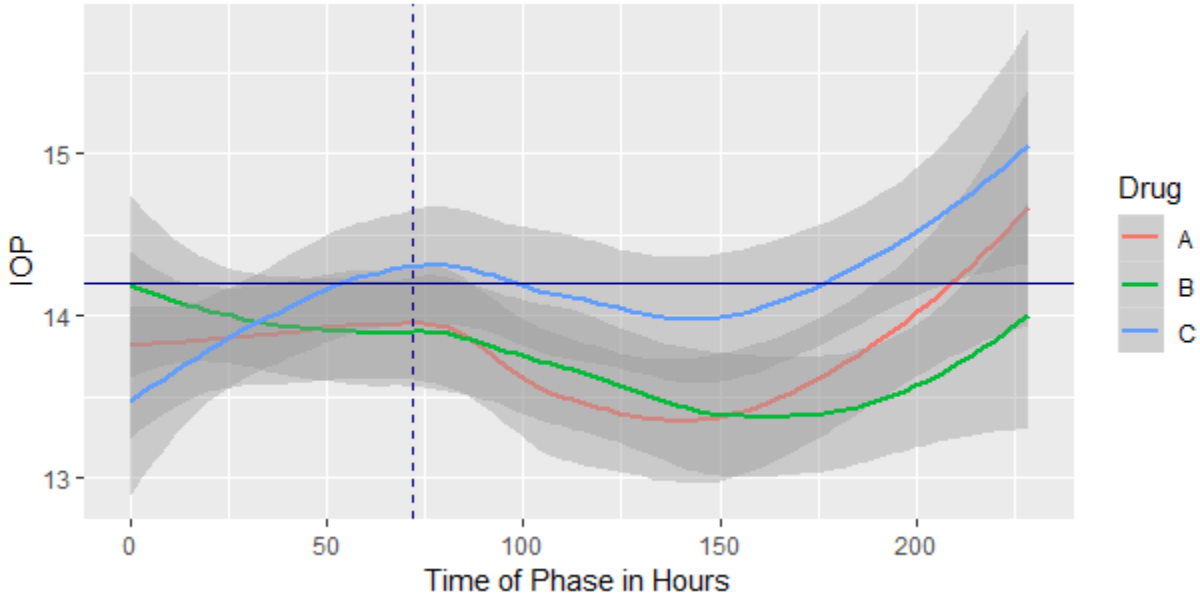


Figure 7. Intra-ocular test results of nine Beagles administered three oral analgesics (gabapentin, tramadol or meloxicam) over a 24-hour period in comparison to baseline

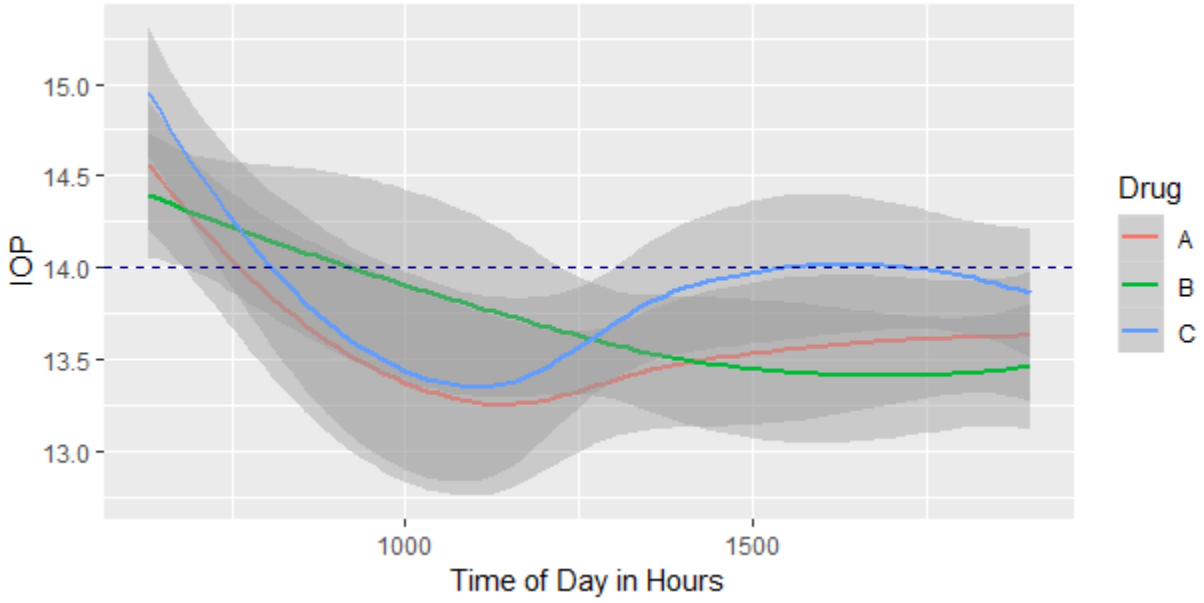


Figure 8. Pupillary diameter results of nine Beagles administered three oral analgesics (gabapentin, tramadol or meloxicam) for the first three days (dashed line) of a 10-day phase in comparison to the baseline value from the acclimation period (solid line)

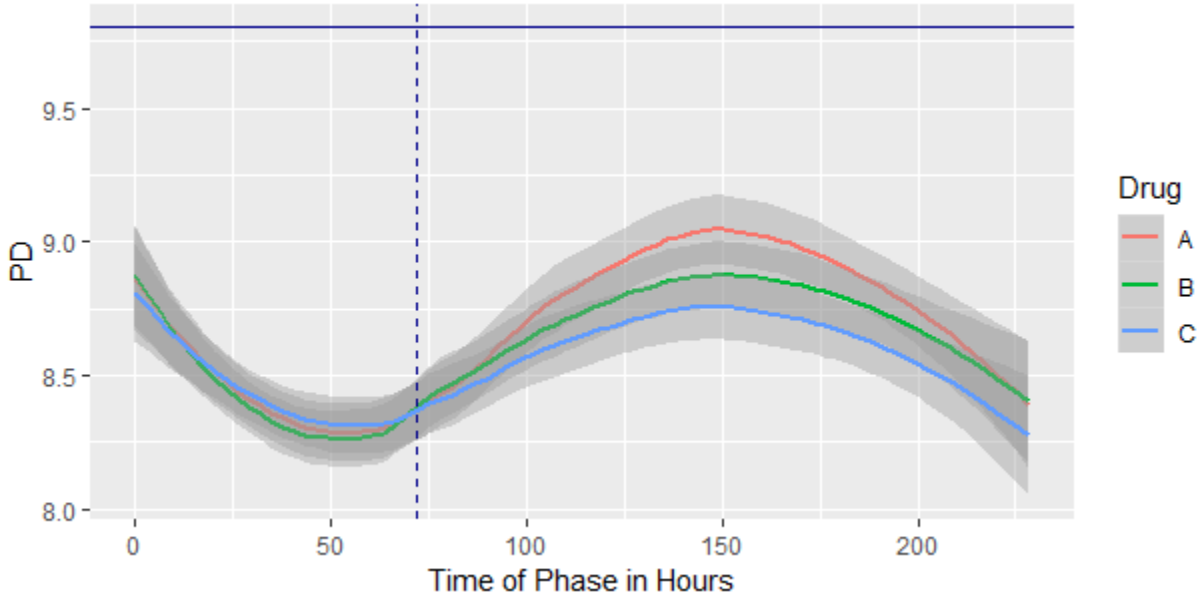


Figure 9. Pupillary dilation test results of nine Beagles administered three oral analgesics (gabapentin, tramadol or meloxicam) over a 24-hour period in comparison to baseline

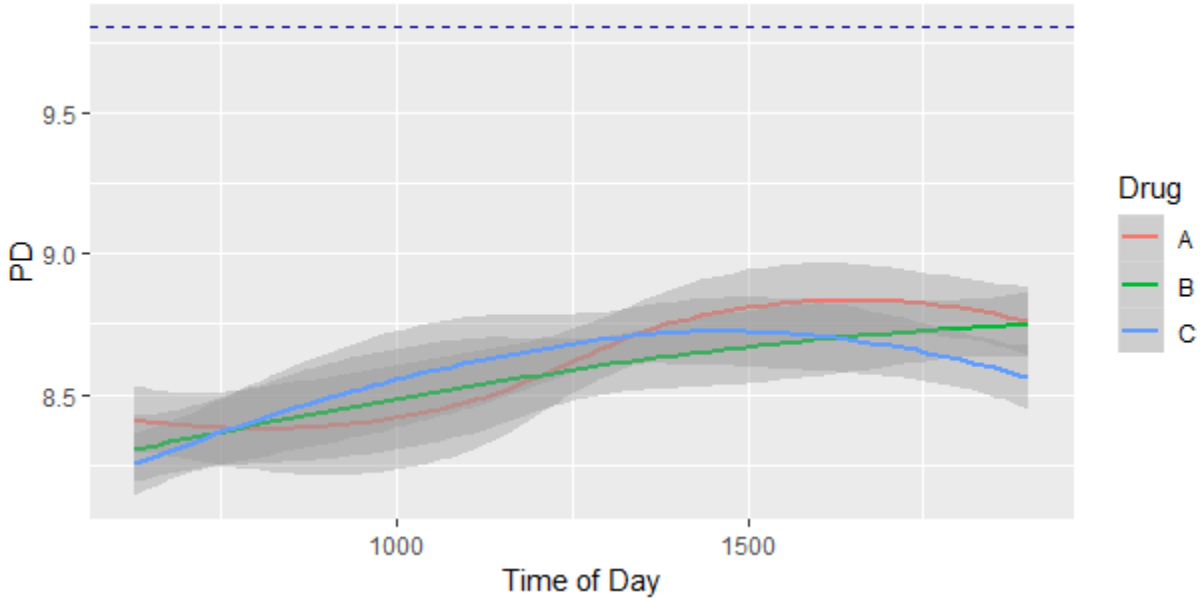


Figure 10. Tear break-up time test results of nine Beagles administered three oral analgesics (gabapentin, tramadol or meloxicam) for the first three days (dashed line) of a 10-day phase in comparison to the baseline value from the acclimation period (solid line)

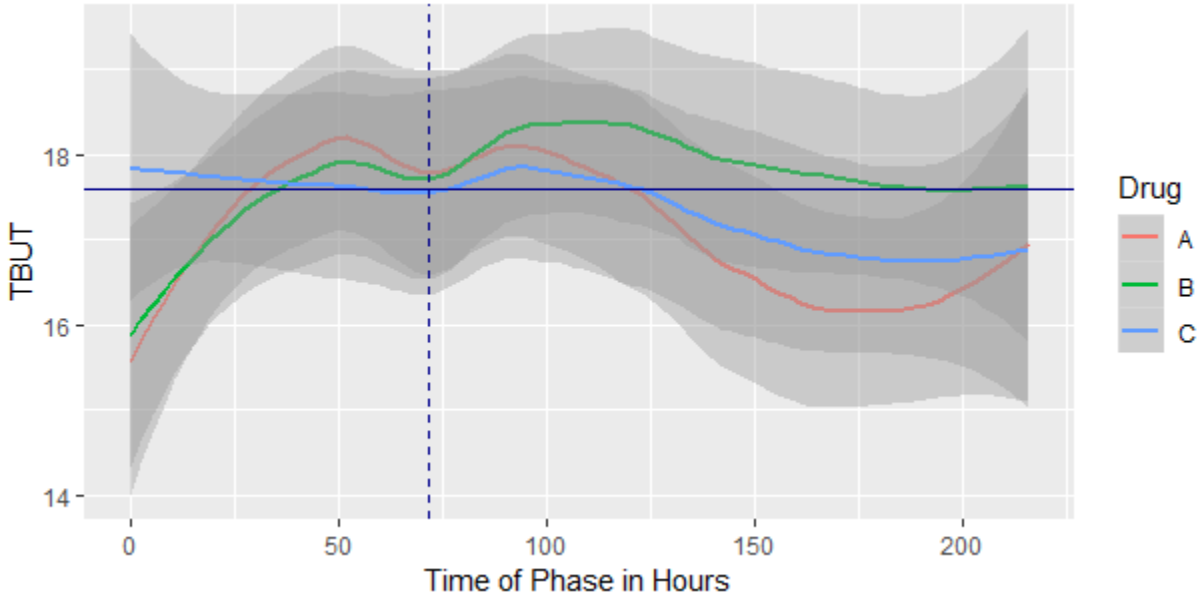
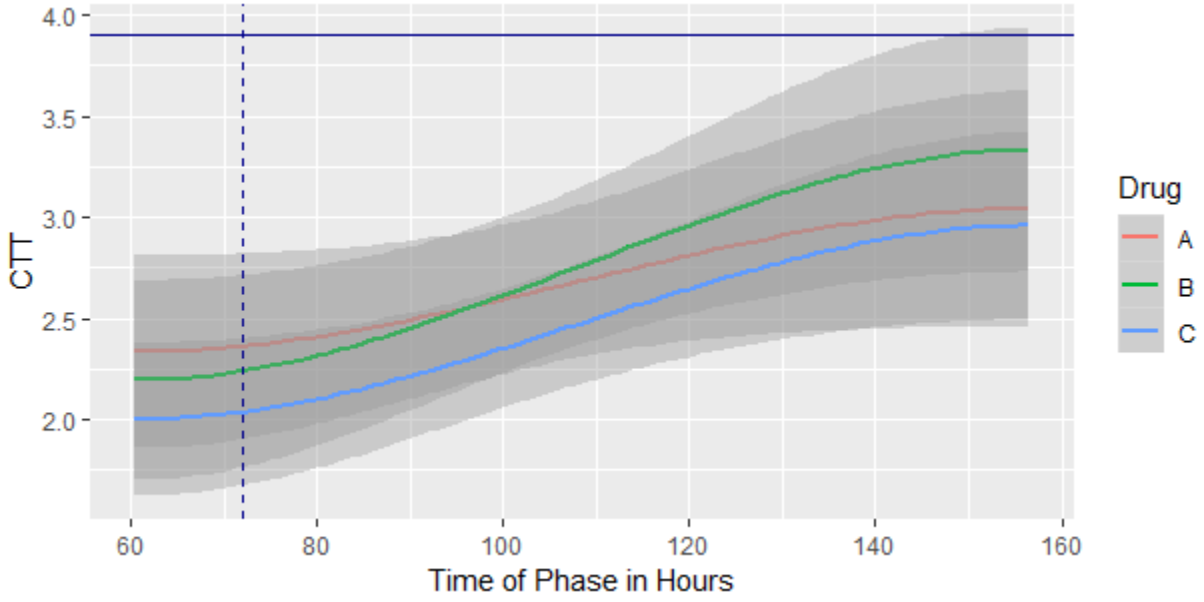


Figure 11. Corneal touch threshold test results of nine Beagles administered three oral analgesics (gabapentin, tramadol or meloxicam) for the first three days of a 10-day phase in comparison to the baseline value from the acclimation period (solid line)



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3 Chapter 3: Summary and Future Directions

Gaining a better understanding of the impact systemic analgesics have on ocular parameters in our canine patients is of paramount importance. As veterinarians, our goals are to do no harm and to have a positive influence on our patients' quality of life. We are also charged with mitigating potentially harmful sequelae any treatment, including medical therapy, may have on a patient. Analgesics are often viewed as having only positive effects on a patient's well-being; however, we must acknowledge that no medication is truly innocuous. Understanding the effect a medication may have on a canine patient's ocular health allows the clinician to make an evidence-based recommendation on whether to prescribe a medication, and which medication is most appropriate in the clinical circumstance. In turn, this enables the client to make an informed decision regarding treatment for their pet. Unfortunately, there is a significant gap in the existing literature regarding the effects of systemic analgesics on ocular health. Available studies have typically evaluated only one or two parameters of ocular health and have examined largely only injectable analgesics[1–9]. Additionally, much of the available literature is riddled with limitations secondary to suboptimal study design, lack of complete and appropriate testing, and failure to conduct appropriate statistical analyses.

Our study focused on evaluating the effects of oral gabapentin, tramadol and meloxicam on several ocular parameters in healthy dogs. Ultimately, analysis of our data revealed that oral doses of 10mg/kg of gabapentin and 3mg/kg of tramadol, resulted in a statistically significant but clinically insignificant decrease in IOP. We attempted to utilize

common, minimally invasive, and accepted ophthalmic measurement techniques in our study. As discussed in Chapters 1 and 2, all methods of testing carry inherent limitations with regards to performance and interpretation. Despite many of these techniques being widely accepted as the gold standard, we must appreciate that there are deficiencies in our ability to accurately evaluate the effect of systemic analgesics on ocular health.

Limitations of our study include few data points when evaluating TBUT and CTT. Additional data points may have allowed us to appreciate subtle changes in these parameters and may have demonstrated a statistically significant difference between treatments. Unfortunately, performing CTT on a daily basis would have been extremely challenging due to the heightened potential for iatrogenic corneal trauma potentially resulting in undue stress and discomfort to the research animals. Our chosen method of evaluating pupillary diameter may be considered a limitation of our study given the extremely subjective nature by which PD is evaluated when handheld calipers are used. Although this method of evaluating PD is widely accepted[10–12], the validity of the measurements obtained, given how rapidly PD diameter can change in both awake and sedated dogs, must be questioned[10,12]. Another potential limitation of our study relates to our use of compounded gabapentin and tramadol. We elected to use liquid, compounded medications to ensure accuracy of dosing. It should be noted that these medications were compounded using published and well established methods[13,14], under the guidance of a registered pharmacist[a]. In retrospect, it would have been advisable to analyze the concentration of the compounded medications used prior to use in the study, to ensure accuracy of the quoted medication doses. Another potential

adjustment to our study protocol could have involved a longer course of therapy for the medications evaluated, as canine patients are typically prescribed analgesia for longer than a three-day period. Lastly, it would have been ideal to have had a more representative canine study population, at minimum, including female dogs in our study population. Unfortunately, a more diverse population of research animals was not available from the supplier, and as such, this aspect of our study was not under the investigators' control.

Based on our results and the available literature, gabapentin is a medication that should be more closely evaluated, whereas previous studies evaluating tramadol and meloxicam have not revealed results that are as promising[1,4,9,15]. Our results revealed a statistically significant, but clinically insignificant decrease in IOP (-0.45mmHg, p-value = 0.038) following oral administration of 10mg/kg gabapentin. It is possible, that at higher doses, or in eyes suffering from ocular pathology, that this decrease in IOP may become clinically significant. If a clinically significant decrease in IOP were noted at higher doses of gabapentin, this analgesic may be a suitable choice for those patients suffering from glaucoma, or for those with a predisposition to develop the disease. Furthermore, topically applied 0.5% gabapentin has also been shown to decrease inflammation in rabbit eyes[16], it is therefore possible that topical application of this medication may decrease ocular pain associated with uveitis in canine eyes. A future study could also involve comparison of gabapentin at different oral doses (10mg/kg versus 20mg/kg) and a topically applied formulation. As gabapentin is a neuropathic pain analgesic, and given how richly innervated the cornea, globe and adnexa are, it is

possible that a topically applied formulation may provide effective analgesia to the corneal surface, and effective anti-inflammatory effects for the eye's anterior segment. Based on the findings published by Anfuso *et al.*, where they showed that topically applied gabapentin resulted in a significant reduction in both clinical signs and biomarkers of inflammation (p-value < 0.05)[16], topically and/or systemically administered gabapentin may prove a useful adjunctive analgesic treatment for inflammatory conditions affecting the canine eye. Additionally, it would be interesting to see whether gabapentin would have any effect on corneal wound healing. If no detrimental impact was noted with regards to healing of the cornea, topically applied gabapentin may provide effective pain relief for those patients suffering from corneal ulcers and concurrent uveitis. This would allow the veterinary practitioner to begin treatment without worry that this would delay corneal would healing, as is the concern with topically applied steroids and non-steroidal anti-inflammatory medications.

In conclusion, our study has shown that gabapentin, tramadol and meloxicam do not appear to have a detrimental impact on canine eyes, when administered at 10mg/kg PO q8, 3mg/kg PO q8, and 0.2mg/kg PO once, followed by 0.1mg/kg PO q24, respectively. Ultimately, our study did not reveal a clinically significant benefit to administration of these medications in healthy eyes; an expected finding as healthy eyes do not require analgesic or anti-inflammatory treatments. Future studies could involve evaluating the effects of these medications in specific ocular disease states (e.g. glaucoma, uveitis, corneal trauma), or evaluation of a single analgesic at different doses and formulations (e.g. gabapentin). Based on results of these studies, synergistic activity of gabapentin

with other analgesics could be evaluated to see whether a statistically and clinically significant impact could be demonstrated on the ocular health of our canine patients.

3.1 Footnotes

a. Personal communication, Heather Kidston Registered Pharmacist, Ontario Veterinary College Health Sciences Centre Pharmacy Manager, Ontario Veterinary College, Guelph, Ontario, Canada

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