Evaluation of systemic absorption of topical ophthalmic prednisolone acetate and dexamethasone in healthy dogs.

by

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Abstract

Objective: To quantify plasma concentrations of prednisolone and dexamethasone following topical ophthalmic application of prednisolone acetate 1% and dexamethasone 0.1% to healthy adult dogs.

Animals: 12 purpose-bred Beagles.

Procedures: Dogs received one drop of either prednisolone acetate 1% (n=6) or neomycin polymyxin B dexamethasone (i.e., dexamethasone) 0.1% (n=6) ophthalmic suspension to both eyes every six hours for 14 days. Blood samples (peripheral and jugular) were collected on day 0, 1, 7, and 14 and analyzed by LC-MS for prednisolone and dexamethasone plasma concentrations. Plasma cortisol levels were measured at the beginning of the study (day 0) and at the end of topical drug administration (day 14).

Results: Both drugs demonstrated systemic absorption. Prednisolone was detected (median; minimum – maximum) on days 1, 7, and 14 (24.80; 6.20 – 74 ng/mL), and dexamethasone was detected (median; minimum – maximum) on days 1, 7, and 14 (2.30; 0 – 17.70 ng/mL). Neither prednisolone nor dexamethasone were detected in plasma samples on day 0 (baseline). Sampling from the jugular vein resulted in higher plasma drug concentrations than peripheral venous samples when samples from each timepoint were combined. Plasma cortisol levels were significantly lower ($P = 0.03$) than baseline following 14 days of treatment with topical prednisolone acetate and dexamethasone.

Conclusions and Clinical Relevance: Prednisolone and dexamethasone are detected in the plasma of healthy dogs following topical ophthalmic administration four times per day. Additional research is needed to evaluate the systemic absorption of topical ophthalmic

prednisolone acetate and dexamethasone in dogs with ocular surface inflammation or anterior uveitis.

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List of Abbreviations

ACTH	Adrenocorticotropic hormone
AUC	Area under the concentration-versus-time curve
$AUC_{0\text{-}6hr}$	Area under the concentration-versus-time curve from time 0 to 6 hours
$AUC_{0\text{-}\infty}$	Area under the concentration-versus-time curve from time 0 to infinity
C_{max}	Maximum observed concentration
HPAA	Hypothalamic pituitary adrenal axis
IS	Internal standard
LC-MS	Liquid chromatography-mass spectrometry
NSAIDs	Non-steroidal anti-inflammatory drugs
T_{max}	Time to maximum concentration
$T_{1/2}$	Half-life
λ_z	Terminal rate constant

Acknowledgements

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Dedication

This report is lovingly dedicated to Kim Griffin. I will forever be grateful to the love and support you provided me. Rest easy, “Mom 2.0”.

Chapter 1 - Background Information and Hypothesis

Treatment of Ocular Surface Inflammation and Anterior Uveitis

Ocular surface inflammation and anterior uveitis are commonly encountered conditions in veterinary medicine that have the potential to be both painful and vision-threatening. Treatment of these conditions often requires anti-inflammatory medications which can be administered topically, subconjunctivally, or systemically. The route of administration of anti-inflammatory medications is determined by the severity and location of the inflammation. Topical ophthalmic glucocorticoids and NSAIDs are most commonly used to control inflammation in the anterior segment of the eye. The topical route of administration is often the preferred route of treatment because of ease of application, high local drug concentration, and minimal systemic side effects.[1] Glucocorticoids are more commonly used than NSAIDs for their greater anti-inflammatory and immunosuppressive efficacy mediated through action on the intra-cytoplasmic glucocorticoid receptor.[2] Glucocorticoids prevent biosynthesis of arachidonic acid by inhibiting phospholipase A_2 and subsequent formation of prostacyclin, thromboxane A_2, prostaglandins and leukotrienes in addition to other inflammatory mediators such as interleukins and tumor necrosis factor alpha.[3] Glucocorticoids decrease prostaglandin synthesis at the level of the cyclooxygenase pathway[4] and may also induce local expression of somatostatin, a cyclic peptide with anti-inflammatory properties.[5] A number of ophthalmic glucocorticoid preparations are available with varying potency, concentration, and degree of corneal penetration.[1,6]

Systemic Absorption of Topical Ophthalmic Medications

When topical ophthalmic medications are applied, they remain within the conjunctival sac for approximately 10 seconds to several minutes depending on the vehicle used, volume instilled, blinking rate, and the degree of reflex tearing elicited.[7,8] A small portion (<5-10%) of

topically applied ophthalmic medications enters the eye after absorption via corneal and, to a lesser extent, non-corneal (conjunctival/scleral) routes.[6,9–13] The majority of the medication is eliminated through the nasolacrimal duct.[7,14] Following topical application of ophthalmic medications, a portion is absorbed systemically via the conjunctiva and/or the nasal, oral, or gastrointestinal mucosa after the medication passes through the nasolacrimal system.[12–18] When absorbed via the ocular, oral, or nasal mucous membranes, medications do not undergo first-pass metabolism in the liver but instead have direct delivery into systemic circulation.[12,19]

Systemic absorption of topically applied ophthalmic glucocorticoids has been evaluated in several species including dogs,[20] horses,[21] rabbits,[22,23] and humans.[24–26] Although systemic absorption is incomplete, systemic side effects including the development of iatrogenic hyperadrenocorticism, suppression of the hypothalamic-pituitary-adrenal axis (HPAA), and glucose dysregulation have been reported following topical application of ophthalmic glucocorticoids.[25–35]

Study Objectives and Hypotheses

Since topical ophthalmic glucocorticoids are commonly prescribed for dogs with ocular surface inflammation and anterior uveitis, knowledge of the systemic absorption of these medications is important due to the potential risk of systemic side effects. The primary aim of this study was to establish the systemic absorption of two commercially available ophthalmic glucocorticoids (prednisolone acetate 1% and neomycin polymyxin B dexamethasone 0.1% ophthalmic suspensions) in healthy dogs. We hypothesized that there would be low, but detectable, plasma concentrations of prednisolone and dexamethasone following topical ophthalmic application.

A secondary objective of this study was to evaluate the potential impact of sampling site on plasma drug concentrations. Previous studies evaluating plasma drug concentrations following oral mucosal administration have found increased drug levels in samples obtained from the jugular vein when compared to samples obtained from peripheral veins.[36–38] Since the jugular veins drain the mucosa from the conjunctiva, nose and oral cavity,[39] we hypothesized that there would be increased plasma drug concentrations in jugular samples when compared to peripheral venous samples.

A tertiary objective of this study was to evaluate for suppression of the HPAA following topical administration of ophthalmic glucocorticoids in healthy dogs. We hypothesized there would be a significant decrease in cortisol levels from baseline following topical ophthalmic application of both prednisolone and dexamethasone.

Chapter 2 - Materials and Methods

Animals Studied

Twelve purpose-bred young adult Beagles owned by the Kansas State University Comparative Medicine Group were enrolled in this study. None of the dogs had received topical or systemic glucocorticoids for at least one month prior to enrollment in the study. All dogs were housed in a climate-controlled environment with a 12-hour light-dark cycle. Dogs were individually housed in separate enclosures for the duration of the study. All protocols were reviewed and approved by the Kansas State University Institutional Animal Care and Use Committee.

Examination

Prior to enrollment, each dog underwent a complete physical and ophthalmic examination performed by both a board-certified veterinary ophthalmologist (AJR) and a resident-in-training (MME). The ophthalmic examination included a neuro-ophthalmic examination (menace response, direct and indirect pupillary light reflexes, and palpebral reflex), measurement of aqueous tear production (Schirmer tear test, Intervet Inc., Summit, NJ, USA), fluorescein staining (fluorescein sodium, Akorn, Inc., Lake Forest, IL, USA), rebound tonometry (Tono-Vet®, Icare Finland, Helsinki, Finland), slit-lamp biomicroscopy (SL-17 portable slit-lamp biomicroscope, Kowa Co, Tokyo, Japan), and binocular indirect ophthalmoscopy (Welch Allyn binocular indirect ophthalmoscope, Welch Allyn Distributors, Skaneateles Falls, NY, USA) following pharmacologic dilation (tropicamide 1% ophthalmic solution, Akorn, Inc., Lake Forest, IL, USA). All dogs had normal ophthalmic and physical examinations prior to inclusion in the study.

Acclimation

The dogs were given a three-day acclimation period prior to the treatment phase of the study. During this acclimation period, each dog was administered one drop of preservative-free artificial tear solution (Refresh Plus® preservative-free lubricant eye drops, Allergan, Inc., Irvine, CA, USA) to each eye, three times daily.

Topical ophthalmic drug administration

The dogs were randomly assigned to receive either prednisolone acetate 1% ophthalmic suspension (prednisolone acetate 1% ophthalmic suspension, Pacific Pharma, Inc., Irvine, CA, USA) or neomycin polymyxin b dexamethasone 0.1% ophthalmic suspension (neomycin polymyxin b dexamethasone 0.1% ophthalmic suspension, Sandoz, Inc., Princeton, NJ, USA), with six dogs in each treatment group. After vigorous shaking of the dropper bottle for approximately 10 seconds, one drop the assigned treatment was applied to each eye four times a day (6 PM, 12 AM, 6 AM, 12 PM) for a total of 14 days (days 1-14) by the same investigator (MME). The dogs were allowed to blink normally after instillation of the eyedrops.

Sample collections

Following the acclimation period (day 0), peripheral venous blood samples were collected from each dog for measurement of plasma drug (prednisolone or dexamethasone) and cortisol concentrations, serving as baseline prior to the treatment phase of the study (Figure 2.1). Fifteen minutes following instillation of the 12 PM treatment on days 1, 7, and 14 of the study period, peripheral venous blood samples were collected from each dog to determine the plasma levels of prednisolone or dexamethasone. Six dogs (three from each treatment group) had jugular venous blood samples collected for plasma drug concentration measurement immediately following the 15-minute peripheral blood collection on days 1, 7, and 14 to assess the effects of

sample site on plasma drug concentrations. In the remaining six dogs (three from each treatment group), peripheral venous samples were collected 30 minutes after the 12 PM treatment on days 1 and 7, and 30, 60, 180, and 360 minutes after the 12 PM treatment on day 14 for plasma drug concentration measurements. All dogs had peripheral venous samples collected 15 minutes following the 12 PM treatment on day 14 for plasma cortisol measurements (Figure 2.1). All blood samples obtained for plasma drug concentrations were collected in lithium heparin tubes. Following centrifugation and plasma separation, samples were frozen and stored in a -80° C freezer until analyzed.

<table>
<tr><td rowspan="8">N = 6</td><td rowspan="4">N = 3</td><td rowspan="4">Preservative free lubricating drop 3 x/day</td><td rowspan="4">Peripheral plasma drug & cortisol concentrations</td><td rowspan="3">30 minute peripheral</td><td rowspan="3">30 minute peripheral</td><td>6 hour peripheral</td></tr>
<tr><td>3 hour peripheral</td></tr>
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<tr><td>15 minute peripheral</td><td>15 minute peripheral</td><td>15 minute peripheral</td></tr>
<tr><td rowspan="2">Acclimation (Days -3 to -1)</td><td rowspan="2">Baseline (Day 0)</td><td>Day 1</td><td>Day 7</td><td rowspan="2">Day 14</td></tr>
<tr><td rowspan="3">N = 3</td><td rowspan="3">Preservative free lubricating drop 3 x/day</td><td rowspan="3">Peripheral plasma drug & cortisol concentrations</td><td>15 minute peripheral</td><td>15 minute peripheral</td></tr>
<tr><td>15 minute peripheral</td></tr>
<tr><td>Jugular</td><td>Jugular</td><td>Jugular</td></tr>
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Figure 2.1 Study timeline for 12 healthy Beagle dogs receiving either prednisolone acetate 1% (n = 6 dogs) or neomycin polymyxin b dexamethasone 0.1% (n = 6 dogs) ophthalmic suspension four times daily for 14 days. All samples obtained on days 1, 7, and 14 were collected after the 12 PM treatment (e.g., at the time of the day 1 blood draw, dogs had already received a total of four applications, evenly spaced every six hours, of assigned drug). The same design was utilized for both medications.

Plasma drug analysis (prednisolone and dexamethasone)

Plasma preparation for prednisolone and dexamethasone included adding 0.1 mL plasma (incurred sample, standard or quality control) with 0.1 mL methanol, 0.1 mL internal standard solution at 50 ng/mL and 0.3 mL of 4% phosphoric acid in water. The internal standards included either prednisolone-d8 or dexamethasone-d5 (Toronto Research Chemicals (North York, ON Canada). The mixture was vortexed and then applied to 96 well solid phase extraction plates (Oasis PRIME HLB µ-elution plates, Waters Corporation, Milford, MA USA) and positive pressure applied using nitrogen. The wells were then washed with 0.3 mL deionized water, followed by 20% methanol in deionized water. The samples were then eluted using 0.05 mL of acetonitrile:methanol (9:1) into a clean collection plate. Deionized water with 0.2% formic acid, 0.05 mL, was then added to the collection plate and sealed with a cap mat. The injection volume was 0.005 mL.

Plasma concentrations of prednisolone and dexamethasone were quantified with LC-MS (Acquity H UPLC and a TQ-S triple quadrupole mass spectrometer, Waters Corporation, Milford MA, USA). The mobile phase consisted of A: 0.1% formic acid in deionized water and B: acetonitrile using a gradient starting at 90% A to 60% A at 5 minutes, then to 0% A at 6 minutes and returning to 90% A at 8 minutes with a flow rate of 0.5 mL/min. Separation was achieved using a 10 X 2.1-mm, 1.8-µM C18 column (Eclipse Plus C18. Agilent Technologies, Santa Clara, CA, USA) maintained at 55° C. The retention times for prednisolone and dexamethasone were 2.74 and 3.42 minutes, respectively. The spectrometric transitions for prednisolone and the IS solution were 361.4→307.2, 325.2, 147.1 (*m/z*, mass-to-charge ratios) and 369.5→332.3, respectively. The transitions for dexamethasone and IS were *m/z* 393.4→373.2, 355.2, 237.1 and 398.5→378.2. The standard curves were linear from 0.1 to 250 ng/mL with the lower limit of

quantification of 0.1 ng/mL. Accuracy and coefficient of variation were determined for 5 replicates each at 0.5, 15, and 150 ng/mL. The interday accuracy for prednisolone was 83.2, 107.4, and 98.7% of the actual value for 0.5, 15 and 150 ng/mL respectively. The interday precision (coefficient of variation) for prednisolone was 13.4, 7.2, 5.7%, at 0.5, 15 and 150 ng/mL respectively. The interday accuracy for dexamethasone was 75.3, 100.5, 104.1 of the actual value for 0.5, 15 and 150 ng/mL respectively. The interday precision (coefficient of variation) for dexamethasone was 12.1, 7.6, 2.8% at 0.5, 15 and 150 ng/mL, respectively.

Pharmacokinetic analysis

Pharmacokinetic analysis of plasma prednisolone and dexamethasone drug levels were determined using commercially available software (PK functions for Microsoft Excel) using non-compartmental methods. The following parameters were generated or calculated: the area under the curve (AUC) from 0 to 240 minutes, which was determined with the linear trapezoidal rule; and the maximum plasma concentration (C_{max}) and time to C_{max} (T_{max}), which were both determined directly from the data. Other pharmacokinetic variables evaluated were the elimination rate constant (λ_z) and elimination half-life ($T_{1/2}$).

Plasma cortisol analysis

Plasma cortisol concentrations were analyzed by use of a chemiluminescence analyzer (Immulite 1000®, Siemens) with a normal reference range of 20 to 160 nmol/L. Sensitivity of the cortisol assay was 5.5 nmol/L.

Statistical analysis

Statistical analyses were performed to compare the drug concentrations obtained in relation to time (in hours and days) and sampling site (peripheral vs jugular). Normality of the data was assessed using the Shapiro-Wilk test. An independent group *t*-test was performed

evaluating weight and age between treatment groups. Due to violation of the normality assumption for several parameters and the small sample size, nonparametric repeated measurements ANOVA (Friedman's test) and post-hoc Dunn's test were used to determine differences in plasma drug concentrations between the different days, time points, and collection sites. Wilcoxon matched-pairs signed rank tests were used to compare paired jugular and peripheral drug concentrations and paired 0.25-hour and 0.5-hour peripheral drug concentrations when data from all days were combined (days 1, 7, and 14). Wilcoxon matched-pairs signed rank tests were also performed to compare cortisol levels at the end of the 14-day treatment period to baseline values for both treatment groups. Mann Whitney test was used to compare the change in cortisol levels over the course of the study between the dexamethasone and prednisolone treated groups. Statistical analyses were performed using commercially available software (GraphPad Prism 9, GraphPad Software, Inc., La Jolla, CA, USA). $P < 0.05$ was considered significant for all comparisons. Data are presented as median (minimum – maximum).

Chapter 3 - Results

Animals

The 12 dogs included eight castrated males and four spayed females aged 6.9 years (6.9 – 7.6 years) and weighing 10.8 kg (9.7 – 14.0 kg). There was no statistical difference in age (P = 0.07) or weight (P = 0.10) between treatment groups.

Systemic absorption

Plasma prednisolone concentrations

No detectable concentrations of prednisolone were found in samples collected at baseline. Prednisolone was detected in plasma of all treated dogs at all measured time points and sampling sites on day 1 (40.65; 10.20 – 74.00 ng/mL), day 7 (26.10; 13.90 – 45.00 ng/mL), and day 14 (19.40; 6.20 – 47.00 ng/mL). Peripheral plasma concentrations of prednisolone 0.25-hour post-treatment on days 1 (30.25; 10.20 – 48.80 ng/mL), 7 (17.80; 13.90 – 28.80 ng/mL), and 14 (18.00; 13.80 – 45.20 ng/mL) were not significantly different ($P \geq 0.14$).

When each day was tested individually, there was no difference ($P \geq 0.28$) in peripheral prednisolone concentrations and paired jugular concentrations for any day. When all days (days 1, 7, and 14) were combined, jugular prednisolone concentrations (44.10; 22.10 – 74.00 ng/mL) were significantly (P = 0.008) higher than peripheral prednisolone concentrations (19.60; 10.20 – 48.80 ng/mL) (Figure 3.1). The median (minimum – maximum) jugular plasma concentrations of prednisolone relative to peripheral plasma concentrations were 154% (97 – 324%) with 6/9 paired measurements being > 115%. All measurements < 115% belonged to the same dog. On day 14, one peripheral plasma concentration (45.20 ng/mL) was greater than the paired jugular plasma concentration (44.07 ng/mL); all other peripheral plasma concentrations were less than the paired jugular plasma concentration.

There was no difference between paired 0.25-hour and 0.5-hour post-treatment peripheral plasma concentrations of prednisolone on days 1, 7, or 14 ($P \geq 0.57$) (Figure 3.2). When the paired 0.25-hour peripheral and 0.5-hour peripheral plasma samples were combined for all three days, the 0.5-hour post-treatment plasma concentration of prednisolone (28.20; 19.40 – 48.70 ng/mL) was significantly higher ($P = 0.008$) than the 0.25-hour post-treatment plasma concentration (19.50; 13.80 – 48.00 ng/mL).

On day 14, the peripheral plasma concentrations were significantly ($P = 0.045$) higher at the 1-hour post-treatment time point (24.40; 20.40 – 27.90 ng/mL) compared to the 6-hour post-treatment time point (7.30; 6.20 – 7.70 ng/mL), but there were no other significant differences in plasma prednisolone concentrations (Figure 3.3).

Plasma dexamethasone concentrations

No detectable concentrations of dexamethasone were found in samples collected at baseline. Dexamethasone was detected in plasma of all treated dogs at all measured time points on day 1 (2.60; 1.40 – 17.70 ng/mL), day 7 (2.75; 1.20 – 12.80 ng/mL), and day 14 (1.90; 0 – 9.90 ng/mL). On day 14, one dog did not have dexamethasone detected in the jugular sample collected immediately following the 0.25-hour post-treatment peripheral blood draw, but dexamethasone was detected in the peripheral sample. Peripheral plasma concentrations of dexamethasone 0.25-hour post-treatment on days 1 (2.35; 1.40 – 2.70 ng/mL), 7 (2.15; 1.20 – 3.30 ng/mL), and 14 (2.05; 0.80– 3.00 ng/mL) were not significantly different ($P \geq 0.43$).

When each day was tested individually, there was no difference ($P \geq 0.11$) in peripheral dexamethasone concentrations and the paired jugular concentrations for any day. When all days (days 1, 7, & 14) were combined, jugular dexamethasone concentrations (7.10; 0 – 17.70 ng/mL) were significantly ($P = 0.008$) higher than paired peripheral dexamethasone concentrations (2.20;

0.80 – 2.90 ng/mL) (Figure 3.1). The median (minimum – maximum) jugular plasma concentration of dexamethasone relative to peripheral plasma concentration was 331% (162 – 1298%), with 8/9 paired measurements being > 115%. All measurements < 115% belonged to the same dog. On day 14, one dog had a peripheral plasma dexamethasone concentration of 0.8 ng/mL, but did not have a measurable jugular plasma dexamethasone concentration.

A significant difference in paired 0.25-hour and 0.5-hour post-treatment peripheral plasma concentrations of dexamethasone was not identified on days 1, 7, or 14 ($P \geq 0.28$) (Figure 3.2). When the paired 0.25-hour post-treatment peripheral and 0.5-hour post-treatment peripheral plasma samples were combined for all three days, the 0.5-hour post-treatment peripheral plasma concentrations of dexamethasone (2.50; 1.95 – 3.45 ng/mL) were significantly higher ($P = 0.035$) than the 0.25-hour post-treatment peripheral plasma concentration (2.40; 1.50 – 2.85 ng/mL).

On day 14, the peripheral plasma concentrations of dexamethasone were significantly ($P = 0.0195$) higher at the 0.5-hour post-treatment time point (2.30; 1.10 = 3.70 ng/mL) compared to the 6-hour post-treatment time point (0.70; 0.20 – 1.20 ng/mL). There were no other significant differences in plasma dexamethasone concentrations (Figure 3.3).

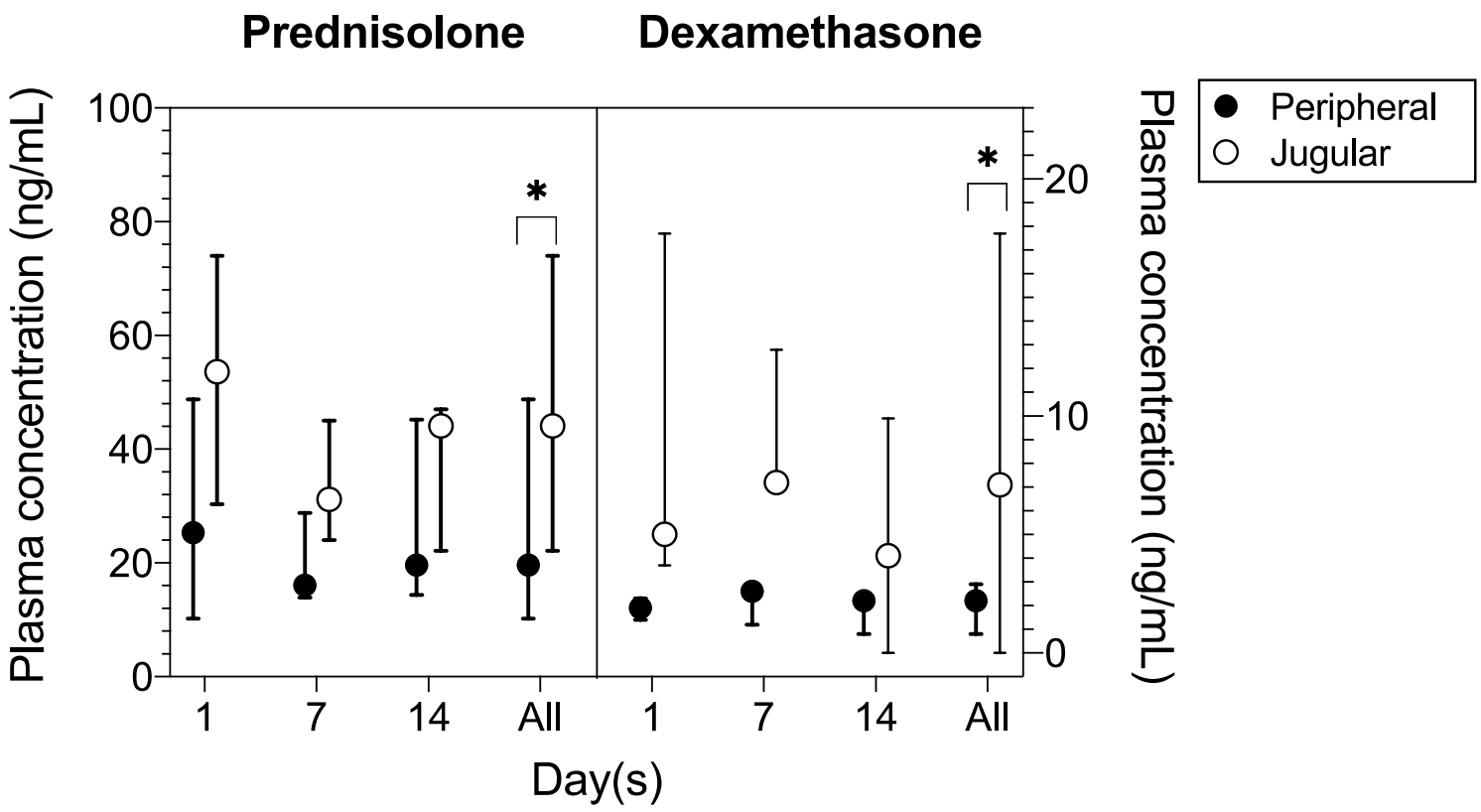

Figure 3.1 Median and range of paired peripheral (closed circle) and jugular (open circle) plasma concentrations of prednisolone (left side) and dexamethasone (right side) after topical ophthalmic application on days 1 (n = 3 paired samples), 7 (n = 3 paired samples), and 14 (n = 3 paired samples), and all days combined (All; n = 9 paired samples). When combined, jugular plasma sample concentrations were significantly higher than peripheral plasma sample concentrations for both prednisolone and dexamethasone (* *P* = 0.008).

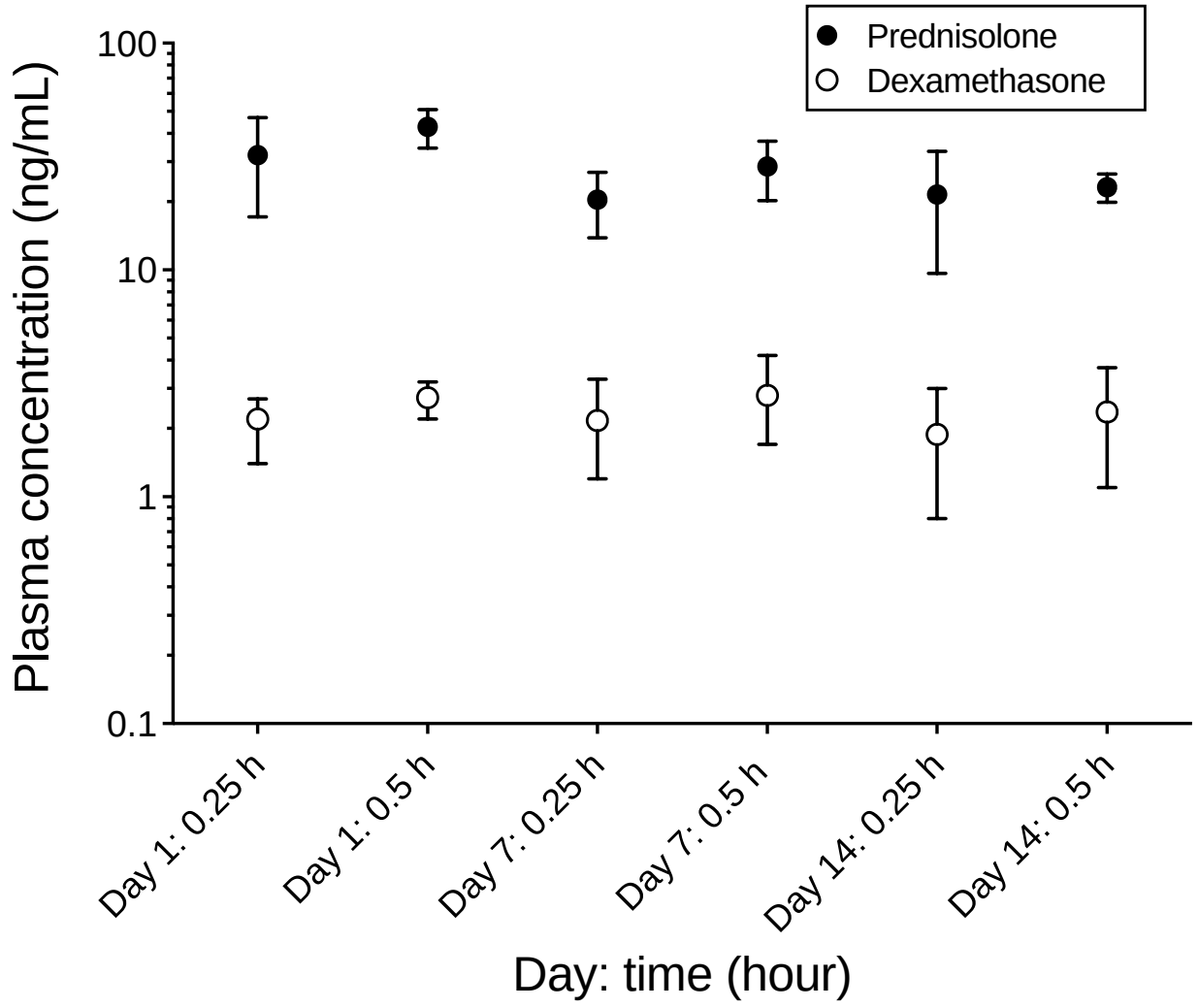

Figure 3.2 Plasma concentrations of prednisolone (closed circle) and dexamethasone (open circle) on days 1, 7, and 14 at 0.25-hour (n = 6 dogs) and 0.5-hour (n = 3 dogs) after topical ophthalmic doses.

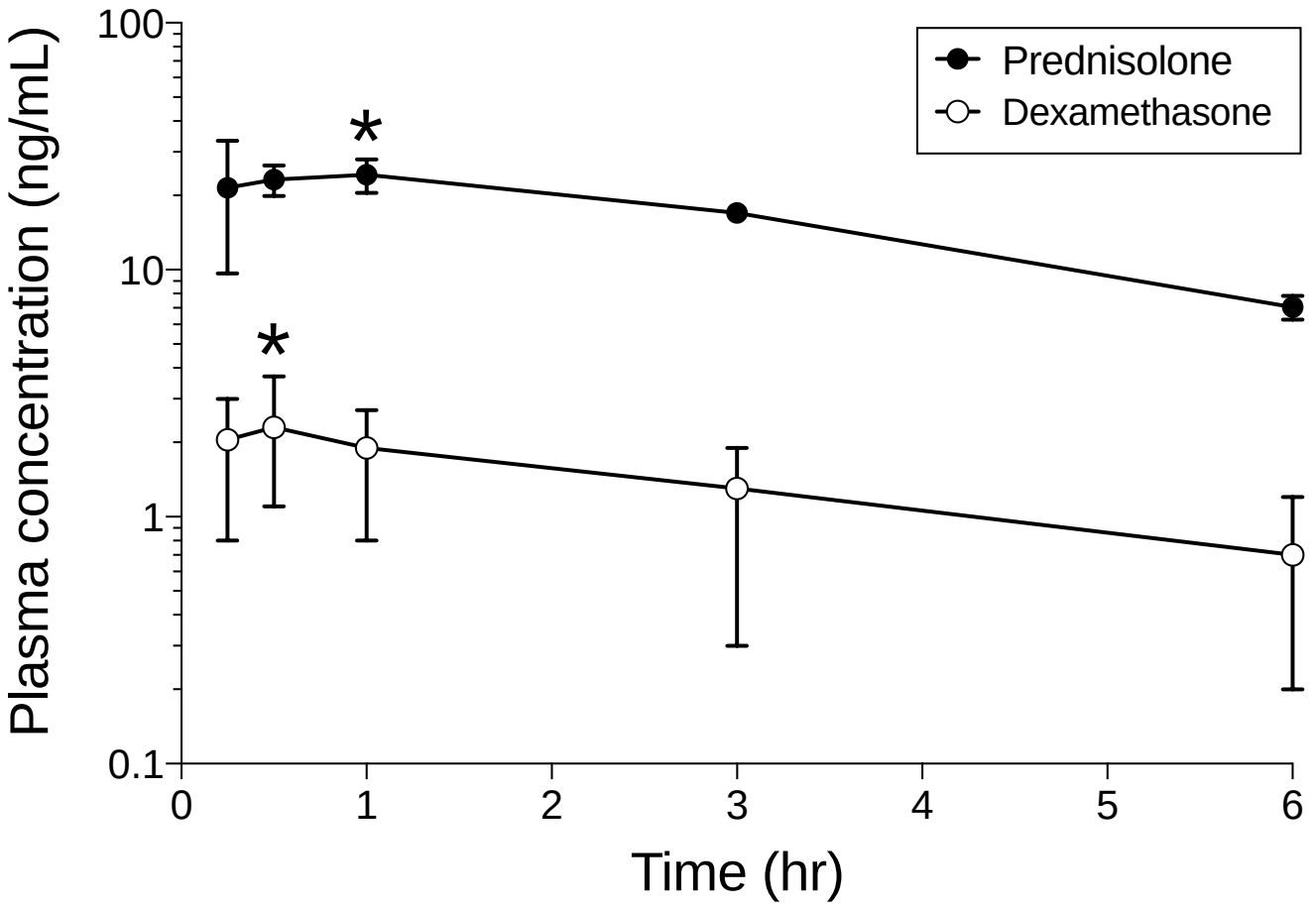

Figure 3.3 Plasma concentrations of prednisolone (closed circle) and dexamethasone (open circle) after the last topical ophthalmic dose on day 14 with n = 6 dogs for the 0.25-hour and n = 3 dogs for the remaining time points. The plasma prednisolone concentrations were significantly (**P* **= 0.045) higher at the 1-hour post-treatment time point (24.40; 20.40 – 27.90 ng/mL) compared to the 6-hour post-treatment time point (7.30; 6.20 – 7.70 ng/mL). Plasma dexamethasone concentrations were significantly (****P* **= 0.0195) higher at the 0.5-hour time point (2.30; 1.10 = 3.70 ng/mL) compared to the 6-hour time point (0.70; 0.20 – 1.20 ng/mL).**

Pharmacokinetic analysis

Following the final topical ophthalmic application of prednisolone or dexamethasone on day 14, the C_{max}, T_{max}, $\lambda_{z,}$, $T_{1/2}$, and AUC from 0 to 240 minutes were determined for three dogs in each treatment group and summarized (Table 3.1).

	AUC_{0-6h} (ng*hr/mL)	**T_{max}** (hr)	**C_{max}** (ng/mL)	**λ_z** (1/hr)	**$T_{1/2}$** (hr)
Prednisolone					
Dog 1	96.5	0.50	24.8	0.233	2.97
Dog 2	87.9	1.00	20.4	0.211	3.29
Dog 3	98.1	1.00	27.9	0.305	2.27
Geometric mean	94.1	0.79	24.2	0.246	2.81
Dexamethasone					
Dog 4	11.9	0.50	3.67	0.162	4.29
Dog 5	2.44	0.50	1.07	0.308	2.25
Dog 6	7.68	0.50	2.29	0.187	3.71
Geometric mean	6.06	0.50	2.08	0.210	3.29

Table 3.1 Pharmacokinetic parameters of prednisolone (n = 3 dogs) and dexamethasone (n = 3 dogs) following the last topical ophthalmic dose (day 14).

Plasma cortisol concentrations

Plasma cortisol levels (median; minimum – maximum) were significantly lower than baseline following treatment with both prednisolone acetate 1% (baseline: 37.50; 18.00 – 44.00 nmol/L vs day 14 of prednisolone treatment: 14.50; 14.00 – 31.00 nmol/L; $P = 0.03$) and

dexamethasone 0.1% (baseline: 50.50; 40.00 – 77.00 nmol/L vs day 14 dexamethasone treatment: 14.00; 14.00 – 19.00 nmol/L; $P = 0.03$).

Chapter 4 - Discussion

This study demonstrates that systemic absorption occurs after topical prednisolone acetate 1% and neomycin polymyxin b dexamethasone 0.1% ophthalmic suspensions are administered to both eyes of healthy dogs four times daily. This study also demonstrates discrepancies in plasma drug concentrations based on blood collection site (jugular vs. peripheral), and significant decreases in endogenous cortisol levels after two weeks of treatment with both of these ophthalmic glucocorticoid suspensions.

Plasma prednisolone concentrations have been previously evaluated in dogs after various routes of administration, including topical ophthalmic[20] and oral.[40] In a recent publication evaluating the tear film pharmacokinetics and systemic absorption of topically applied prednisolone acetate 1% ophthalmic suspension in healthy dogs, the plasma prednisolone concentration ranged from 3.9 to 34.0 ng/mL 10 to 15 minutes following application of one (35 µL) or two drops (70 µL) to each eye four times daily for three days.[20] Similarly, in the present study, the plasma concentrations of prednisolone 15-minutes post-treatment on days 1, 7, and 14 ranged from 10.20 – 48.80 ng/mL. Slight differences in plasma concentrations could be due to interdog variability, lack of dose uniformity in drop size, differences in composition of the commercial drug preparations, or methods used to determine plasma concentrations. While it would have been more precise to administer each drop with a micropipette as was performed in the Sebbag study,[20] this was not performed in the present study in an effort to closely mimic the clinical setting. Both studies utilized generic formulations of prednisolone acetate 1% ophthalmic suspension, and there are variable reports on the dose uniformity of different formulations of prednisolone acetate ophthalmic suspension.[41,42] One study[41]concluded that generic prednisolone acetate had highly variable drug concentrations ranging from 7% to 231.5% of the declared

concentration, while less variability was seen with branded prednisolone acetate (20.5% to 181.4%). A second study[42] did not appreciate this variability as long as bottles containing the suspension were shaken for five seconds prior to dispensing.

The pharmacokinetics following oral administration of prednisolone in healthy dogs have also been described.[40,43] The mean $AUC_{0\text{-}inf}$ following oral administration of prednisolone at 1 mg/kg and 2mg/kg in Beagles was 937.09 ng*hr/mL[40] and 2090.31 ng*hr/mL,[43] respectively. In the present study, the geometric mean $AUC_{0\text{-}6hr}$ (equivalent to AUC_{inf} because prednisolone is at steady state) was 94.05 ng*hr/mL. Given the AUC represents the total drug exposure and should be equivalent to dose, the dogs in the present study had an equivalent prednisolone dose of 94.05/937.09 or 0.1 * 1 mg/kg and 94.05/2090.31 or 0.045 * 2mg/kg (prednisolone doses from published studies[40,43]). This would give a relative exposure comparable to 0.09 to 0.1 mg/kg oral prednisolone. Given that the topical ophthalmic treatments were provided four times daily in the present study, the topical dose would be equivalent to 0.36 to 0.4 mg/kg/day of oral prednisolone, close to what is considered a physiologic dose of prednisolone. The half-life of prednisolone in this study was longer than the 2 mg/kg oral study[43] (2.8 hr vs 1.5 hr), but comparable to the 1mg/kg oral study.[40] The variability between the studies could be due to random variation, interdog differences, concentration dependent differences, differences in absorption rate or differences in study design.

Systemic absorption of topically applied ophthalmic dexamethasone has been evaluated in several species. Dexamethasone was detected in horse serum and urine 10 to 15 minutes following topical application of dexamethasone 0.1% ophthalmic ointment (100 mg) to one eye four times daily for eight consecutive days, and serum concentrations ranged from 0.10 to 0.49 ng/mL.[21] Following frequent application of topical ophthalmic dexamethasone disodium

phosphate (an aqueous solution) in humans prior to vitrectomy, systemic absorption was low, with a mean of 0.7 ng/mL from three to 101 minutes following topical application.[24] Venous blood concentrations of dexamethasone following topical ophthalmic, intranasal, and intravenous administration of 0.5% dexamethasone-cyclodextrin in rabbits was found to be similar at 20-30 ng/g, regardless of the route of administration.[23] To the authors' knowledge, this is the first study to report the systemic absorption of dexamethasone following topical ophthalmic application in dogs. While the plasma concentrations were low, all but one sample was above the lower limit of quantification (0.1 ng/mL). The peripheral plasma concentrations of dexamethasone 15-minutes post-treatment ranged from 0.8 – 3.3 ng/mL.

The pharmacokinetics following intravenous dexamethasone administration in healthy dogs have also been described.[44,45] The mean AUC following intravenous administration of dexamethasone at 0.01 mg/kg and 1 mg/kg in dogs was 2060 ng*min/mL[44] and 155956 ng*min/mL,[45] respectively. In the present study, the geometric mean $AUC_{0\text{-}6hr}$ following topical ophthalmic administration of dexamethasone was 6.036 ng*hr/mL (277 ng*min/mL). The dogs in the present study had an equivalent dexamethasone dose of 0.13 * 0.01 mg/kg and 0.0018 * 1 mg/kg (dexamethasone doses from published studies[44,45]) . This would give a relative exposure comparable to intravenous dexamethasone administration at 0.0013 to 0.0018 mg/kg. Given that the topical ophthalmic treatments were provided four times daily in the present study, the topical dose would be equivalent to 0.0052 to 0.0072 mg/kg/day of intravenous dexamethasone. The half-life of dexamethasone in this study was shorter than the 0.01 mg/kg intravenous study[44] (~2 hr vs 3 hr), but comparable to the 1mg/kg intravenous study.[45]

In the current study, when comparing the peripheral plasma concentrations of prednisolone and dexamethasone following topical ophthalmic suspension administration, the

median concentration of prednisolone (20.4 ng/mL) was 9.95-fold greater than the median concentration of dexamethasone (2.05 ng/mL). Given the concentration of prednisolone acetate 1% ophthalmic suspension is 10-fold that of the neomycin polymyxin b dexamethasone 0.1% ophthalmic suspension, these results suggest equivalent systemic absorption of both topical ophthalmic steroid medications. The conclusion that there is equivalent systemic bioavailability relies on several assumptions, including uniform drop volume and consistency and equal volume of distribution.

A previous study[36] evaluating plasma drug concentrations following sublingual administration in dogs found the jugular sample to have a 4.3-fold higher C_{max} and 2.2-fold higher AUC compared to the saphenous vein, raising concerns for using the jugular vein as a site of sampling for pharmacokinetic studies after transmucosal routes in the head region. In the present study, the jugular concentrations were overestimating the peripheral venous concentrations by a median of 154% for prednisolone and 333% for dexamethasone. This finding supports the conclusion that sampling from the jugular vein will overestimate the systemic bioavailability of a substance that has been absorbed through the mucous membranes of the head.

In a clinical setting, treatment of anterior uveitis can be several months in duration, and in some cases, medication may be necessary indefinitely. Additionally, concurrent treatment with systemic NSAIDs may be indicated, and if there is significant systemic absorption of topical glucocorticoids, an adverse drug reaction could occur. The degree to which different ophthalmic glucocorticoids are absorbed systemically may influence a veterinarian's decision on which medications to use to treat anterior uveitis, especially in small dogs with hyperadrenocorticism or preexisting liver disease. Suppression of the HPAA has been reported following long term use of

difluprednate 0.05% ophthalmic emulsion[46] and prednisolone acetate 1% ophthalmic suspension for two weeks in dogs.[28,29] Topical application of dexamethasone suspension 0.1% four times daily to both eyes in Beagles also resulted in adrenal suppression as well as histopathologic changes in the liver.[30] Reversible iatrogenic hyperadrenocorticism has been caused by the use of a topical ophthalmic glucocorticoid medication in a dog[32] and in humans.[33,47] Unlike in dogs,[48] topical ophthalmic glucocorticoids have been found to significantly increase blood glucose levels in diabetic humans[34,49] undergoing cataract surgery.

Consistent with previous reports,[28–30] the cortisol levels in the present study were significantly ($P = 0.03$) decreased from baseline following four times daily treatment with prednisolone acetate 1% and dexamethasone 0.1% ophthalmic suspensions for two weeks. The percentage reduction of cortisol from baseline ranged from 22 to 70% and 65 to 82% for the prednisolone and dexamethasone treated groups, respectively. Due to the high degree of structural similarity between prednisolone and cortisol, there is approximately 49% cross-reactivity of the cortisol assay (Immulite 1000®, Siemens) with prednisolone, potentially underestimating the degree of cortisol suppression. The clinical significance of the reduced cortisol concentrations and suppression of the HPAA in the present study is difficult to assess as dynamic testing to stimulate production of ACTH or cortisol were not performed. Partial adrenal suppression, characterized by decreased plasma cortisol concentrations with an intact HPAA response to metyrapone tartrate, has been documented following six weeks of topical ophthalmic 0.1% dexamethasone sodium phosphate in humans.[50] A study[51] evaluating the utility of baseline cortisol measurements for the diagnosis of hypoadrenocorticism found that baseline cortisol concentration of ≤ 22 nmol/L have a sensitivity of 96.9% and specificity of 95.7% for hypoadrenocorticism in dogs. In the present study, 16.7% of the baseline cortisol samples were

≤ 22nmol/L. Alternatively, 91.7% of the cortisol samples were ≤ 22 nmol/L following two weeks of four times daily treatment with either topical ophthalmic prednisolone acetate 1% or neomycin polymyxin b dexamethasone 0.1% ophthalmic suspensions.

The results of this study should be interpreted in view of a few limitations. The sample size was low, particularly for evaluation of sample site comparisons and pharmacokinetic parameters, making the study prone to Type II error. Additionally, while blood was sampled from the different sites within the shortest time possible for paired jugular and peripheral samples, blood sampling was not actually simultaneous. For the paired peripheral and jugular samples, the peripheral venous sample was always obtained immediately prior to the jugular sample. The slight delay in obtaining the jugular blood sample could explain the two jugular samples which had lower plasma drug concentrations than the paired peripheral sample. Alternatively, those samples in which the jugular concentration was lower were within the normal analytical variation in plasma drug concentrations. An additional limitation of this study was the method by which suppression of the HPAA was assessed and lack of a negative control group. While there was a significant decrease in cortisol concentrations for both treatment groups, measurement of basal plasma cortisol concentrations alone has limitations as an indicator of adrenal suppression.[52] In normal dogs, serum cortisol concentrations fluctuate episodically throughout the day and can occasionally decrease to subnormal values. Several studies[53–58] have been performed evaluating basal plasma or serum cortisol concentrations in normal dogs with a wide range of reported values (24.8 to 85.5 nmol/L,[53] < 3 to 77.5 ng/mL,[54] 2.29 to 28.20 ng/mL,[55] 16.3 to 27.7 ng/mL,[56] 9.4 to 37 ng/mL,[57] 6 to 28.5 ng/mL[58]). An ACTH-stimulation test ideally would have been performed to determine the integrity of the HPAA. While a negative control group may be beneficial in comparing cortisol levels, it would not add information

regarding the pharmacokinetics aspect of this study and it would have increased animal use in the study by six dogs.

Chapter 5 - Conclusions

The results of this study demonstrate that prednisolone and dexamethasone are detected in the plasma of healthy dogs following topical ophthalmic administration four times per day. Blood samples collected for evaluation of systemic absorption of medications following topical ophthalmic administration should be obtained from peripheral veins so as to not overestimate the systemic bioavailability of a medication. Additionally, topical ophthalmic administration of prednisolone acetate 1% and neomycin polymyxin b dexamethasone 0.1% ophthalmic suspensions administered four times daily for two weeks leads to a significant decrease in endogenous cortisol levels. Additional research is needed to evaluate the systemic absorption of topical ophthalmic prednisolone acetate and dexamethasone in dogs with ocular surface inflammation or anterior uveitis.

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