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Effects of 5% sodium chloride ophthalmic ointment on thickness and morphology of the normal canine cornea

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Abstract

Objective—To determine the effect of 5% sodium chloride ophthalmic ointment (5% NaCl) on thickness and morphology of the normal canine cornea using ultrasonic pachymetry (USP), *in vivo* confocal microscopy (IVCM), and Fourier-domain optical coherence tomography (FD-OCT).

Methods—Five healthy laboratory Beagles received ophthalmic examinations including USP, IVCM, and FD-OCT prior to and at fixed intervals following treatment. The right and left eyes were treated with 5% NaCl and artificial tears ophthalmic ointment (AT), respectively, every 2 h for 4 treatments/day (days 2–9); and then hourly for 7 treatments/day (day 10). Treatment groups were statistically compared using mixed effects linear regression.

Results—Treatment with 5% NaCl resulted in a 12 μm decrease in corneal thickness from baseline ($P < 0.001$), while there was no significant difference in corneal thickness between values obtained at baseline and following treatment with AT ($P = 0.82$). Epithelial cell density significantly increased from baseline (530 ± 52 cells/ mm^2) to 577 ± 43 and 567 ± 15 cells/ mm^2 with 5% NaCl and AT, respectively ($P = 0.003$ and 0.005 , respectively). However, keratocyte cell density in the anterior and posterior stroma and endothelial cell density did not significantly differ following treatment with 5% NaCl or AT ointment ($P > 0.05$).

Conclusions—Short-term topical treatment with 5% NaCl decreased corneal thickness in normal dogs with no observable changes in corneal morphology or signs of ocular toxicity.

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Keywords

Cornea; 5% sodium chloride ophthalmic ointment; ultrasonic pachymetry; optical coherence tomography; in vivo confocal microscopy; canine; corneal edema

Introduction

Corneal transparency is essential to normal functioning of the eye. Contributing to the maintenance of corneal transparency are the presence of small, uniformly sized collagen fibrils organized into well defined fibers and lamellae and maintaining a relative state of stromal deturgescence.^{1,2} Corneal hydration and stromal thickness are intimately linked. A relatively dehydrated stroma is maintained under normal conditions through both barrier functions and active transport processes associated with the anterior corneal epithelium and the posterior corneal endothelium, respectively.³ Of these two, the endothelium plays a more significant role as complete removal of epithelium results in increasing corneal thickness by 200% while removal of the endothelium results in a 500% increase in corneal thickness.⁴ Any appreciable degree of corneal edema is associated with an increase in corneal thickness, reduction in transparency and vision compromise. Persistent, severe, diffuse corneal edema can also result in conditions associated with marked ocular discomfort such as bullous keratopathy, corneal ulceration, and infection.

Osmotic agents are the most commonly employed topical medications in physician-based ophthalmology to palliatively address corneal edema, with several studies documenting superior efficacy of 5% sodium chloride ophthalmic ointment (5% NaCl) in comparison to other hyperosmotic agents including colloidal dextran polysaccharide, glycerine, sodium sulfacetamide solution, gum cellulose, and Karo syrup.^{5–11} A study in normal rabbits evaluated various hypertonic solutions and found that solutions containing 2 or 5% NaCl combined with a patented water-soluble polymer (Adapt®) produced corneal thickness reductions of approximately 8 to 10%.⁶ In human patients with bullous keratopathy, 5% NaCl solution was effective in improving visual acuity and decreasing corneal thickness in early but not advanced cases.⁸ Another study advocated the use of frequent administration (every 2 h during waking hours) of 5% NaCl in combination with a hydrophilic contact lens for bullous keratopathy and an improvement in visual acuity was noted in some patients.¹² To the authors' knowledge, the efficacy of osmotic agents for corneal edema in dogs has not been evaluated although anecdotal reports suggest that 5% NaCl may decrease corneal thickness and/or reduce bullae formation.

While *in vivo* advanced imaging techniques are commonplace in physician-based ophthalmic practice for the management of corneal disorders, ultrasonic pachymetry (USP), Fourier domain-optical coherence tomography (FD-OCT), and *in vivo* confocal microscopy (IVCM) are rarely used for the diagnosis and treatment of corneal disorders in canine patients^{13–17} although normative data exists.^{18–23} Thus, the purpose of this study were to determine the effects of 5% NaCl ophthalmic ointment on corneal thickness in normal beagles using USP and FD-OCT and corneal morphology using IVCM and FD-OCT.

Methods

Animals

A paired t-test power analysis showed that 5 dogs would allow us to detect a 5% difference in central corneal thickness between eyes with a power of 0.8 and an alpha of 0.05; a previously reported CCT in normal Beagles was used for this analysis.¹⁸ Thus, five healthy Beagle female dogs (10 eyes) with a mean age of 1.82 ± 0.01 years and weight of 7.58 ± 0.50 kg were included in the study. Examination prior to and at fixed time points after treatment included the following procedures in order of when they were performed: Schirmer tear test (Schering-Plough Animal Health, Union NJ 07083), color digital single lens reflex (SLR) photography with a Nikon D100 (Nikon Inc., Tokyo, Japan) camera attached to a Nikon autofocus micro Nikkor 105 mm (1:2.8 D) lens and a Nikon macro speedlight SB-29s ring flash, slit lamp biomicroscopy (SL-15 Slit-Lamp, Kowa Optimed, Inc., Torrance, CA, USA), binocular indirect ophthalmoscopy (Vantage Plus Wireless, Keeler Instruments Inc., Broomall, PA, USA) using a 28D indirect lens (Volk Optical, Inc., Mentor, OH, USA), ultrasonic pachymetry (USP; Accupach VI; Accutome Ultrasound Inc., Malvern, PA, USA and/or Pachette 3; DGH Technology, Inc., Exton, PA, USA), FD-OCT (RTVue® 100, software version 6.1; Optovue Inc., Fremont, California, USA), IVCN (ConfoScan 4; Nidek Technologies, Gamagori, Japan), digital slit lamp photography (Imaging Module IM 900; Haag Streit, Koeniz, Switzerland), rebound tonometry (TonoVet, Jorgensen Laboratories, Inc., Loveland, CO, USA), and fluorescein stain (HUB Pharmaceuticals, LLC, Rancho Cucamonga, CA, USA), and External photography was then performed after topical fluorescein stain application with a Nikon D300 SLR digital camera with BLUE-AWB Wratten gelatin filters (Kodak, Rochester, NY, USA) placed over the twin lights (Nikon wireless remote speed light SB-R200) and a yellow barrier filter over the lens (HOYA HMC 62mm Y [k2] filter, Tokyo, Japan). The ambient temperature (21 ± 2 °C) and light-to-dark cycle (14 h of light to 10 h of darkness) of the housing area were controlled. All dogs were housed separately in the same room and had ad libitum access to fresh water and a commercially prepared diet.

Dogs were sedated with dexmedetomidine (2.5–5 µg/kg) intramuscularly (IM) or intravenously (IV) for 30–45 min to facilitate imaging; an equal volume of atipamezole was administered IM to reverse the sedation at the completion of imaging. Dogs were then placed in sternal recumbency and an isotonic buffered ophthalmic solution (OcuSOFT Eyewash, Altaire Pharmaceuticals, Inc., Aquebogue, NY, USA) was applied to prevent corneal desiccation as needed throughout the sedation and subsequent procedures.

All aspects of the study were approved by the Institutional Animal Care and Use Committees of the University of California-Davis (#16547) and were performed according to the Association for Research in Vision and Ophthalmology resolution on the use of animals in research.

Study Design

One day following baseline examination (day 2), each dog received 0.05 ml of commercially available 5% NaCl ointment (Akorn, Inc., Lake Forest, IL, USA) applied to the superior

bulbar conjunctiva of the right eye (OD; treated eye). An equal amount of artificial tear (AT) ointment (Akorn, Inc., Lake Forest, IL, USA) was applied to left superior bulbar conjunctiva (OS; control eye). Ointments were preloaded into tuberculin syringes and stored and applied at an ambient temperature of 21 ± 2 °C to ensure uniformity of ointment delivery. On days 2 through 9, dogs received 5% NaCl OD and AT OS every 2 h for 4 treatments/day; treatments were then administered hourly for 7 treatments on day 10. Treatments were begun at 8 am daily and administered at the same time daily to minimize diurnal variations in corneal thickness.²⁴ Accupach and/or Pachette USP measurements were obtained prior to treatment application and then performed 30 min after each treatment application on days 1–10; FD-OCT was performed on day 1, 7, and 10; and IVCN was performed on days 1 and 10. Following initiation of treatment, the FD-OCT and IVCN were performed 2 h after the last application of ointment.

Clinical assessment

Ultrasonic pachymetry was performed on both eyes of all dogs in the central, superior, inferior, nasal and temporal perilimbal cornea as previously described.¹³ If a measurement could not be obtained with the Pachette, then the Accupach was substituted and/or the cornea was rinsed with OcuSOFT Eyewash (Altaire Pharmaceuticals, Inc., Aquebogue, NY, USA) or 5% NaCl ophthalmic solution (Akorn, Inc., Lake Forest, IL, USA).

The FD-OCT imaging (26000 A scan/sec, 5 µm axial resolution, 840 nm superluminescent diode) of the central cornea was performed in both eyes of all dogs using previously described methods.¹³ The RTVue measuring tool was used to measure full thickness of the cornea as well as thickness of the epithelium, stroma and Descemet's membrane (DM)-endothelium complex.

The IVCN with a 40x/0.75 objective lens was used to image the central cornea of each eye of all dogs as previously described.¹³ Manual cell counts were performed using the ConfoScan 4 NAVIS imaging software. Three images per location were analyzed and these results were averaged. For all cell counts, the cells touching the borderlines were counted only along the upper and right border. Those cells touching the left and lower border were omitted from analysis.

Ophthalmic examinations were performed and clinical assessment was performed using the semiquantitative preclinical ocular toxicology scoring (SPOTS) system prior to initiating the study and 2 h after the final topical treatment each day; the SPOTS system was used to assess the pupillary light reflex, conjunctival hyperemia, conjunctival swelling, conjunctival discharge, corneal opacity (severity and area), corneal vascularization, anterior chamber (AC) flare, AC cell, iris involvement, anterior vitreous cell, fluorescein stain (severity and area), lens opacity, vitreous haze, and degraded fundus view.²⁵ Finally, the corneas were stained with fluorescein to determine if an epithelial defect was present.

Statistics

For corneal thickness measurements, mixed effects linear regression was used to evaluate the main effects of eye, method of measurement, day and time, and the interaction effect of treatment and location. For central corneal thickness, mixed effects linear regression was

used to evaluate the main effects of eye, method of measurement, day, time and treatment. For cell density, mixed effects linear regression was used to evaluate the main effects of eye and treatment. For the aforementioned analyses, the individual dog was treated as a random effect and an identity covariance structure was used; all other variables were considered as fixed effects. Post-hoc tests were performed using a Bonferroni-adjusted p -value to preserve a nominal type 1 error rate of 0.05. Analyses were performed using Stata/IC 12.1 (StataCorp LP, College Station, Texas, USA). Least squares linear regression was used to assess correlation between measurements obtained with the Accupach and Pachette. All measurements were expressed as mean $\pm$ standard deviation (SD).

Results

Clinical assessment

In general, topical treatment was well tolerated by patients. Findings using the SPOTS system²⁵ included mild conjunctival hyperemia in all dogs following the last USP measurement of each day. Moderate conjunctival hyperemia was noted in three to four subjects on days 7, 9, and 10. In all subjects, bilateral mild diffuse multifocal punctate erosions with subtle fluorescein stain retention was noted following the last USP measurement of each day (Figure 1). One subject developed a punctate superficial epithelial defect OD as evidenced by fluorescein stain retention on day 3 that had resolved by the following day.

Corneal thickness measurements using USP and FD-OCT

Both the Pachette and Accupach were utilized as there was difficulty acquiring data points at the later time points daily with the Pachette presumably due to accumulation of debris from ointment application that led to interference with acquisition of readings. This problem was minimized with frequent cleaning of the probe tip but 30% of the readings required the Accupach to be substituted for the Pachette. Of the 2457 corneal thickness measurements acquired throughout the study, a corneal rinse with OcuSOFT Eyewash or 5% NaCl ophthalmic solution for 41 (1.6%) and 3 (0.12%) measurements, respectively, was necessary to remove accumulated debris to allow USP to be performed. There was no significant difference in corneal thickness measurements obtained using USP-Accupach or USP-Pachette ($P = 0.593$) and good correlation was observed ($R^2 = 0.67$) between these two ultrasonic pachymeters (Figure 2). By contrast, corneal thickness measurements obtained by automated and manual FD-OCT pachymetry were significantly less by 10 ($P = 0.019$) and 19 μm ($P < 0.001$), respectively, in comparison to USP-Accupach. Similarly, measurements obtained by automated and manual pachymetry were significantly less by 11 ($P = 0.012$) and 19 μm ($P < 0.001$), respectively, in comparison to USP-Pachette. There was no significant difference in corneal thickness measurements obtained by automated or manual FD-OCT pachymetry ($P = 0.319$). Using USP-Pachette and manual FD-OCT, central corneal thickness (CCT) prior to treatment did not significantly differ between eyes at 551 ± 22 and $529 \pm 26 \mu\text{m}$ for the left eye and 560 ± 22 and $520 \pm 31 \mu\text{m}$ for the right eye, respectively ($P = 0.091$). Using USP-Pachette prior to treatment, the central cornea was significantly thinner than the nasal, temporal, superior and inferior cornea at 556 ± 20 , 585 ± 20 , 591 ± 24 , 610 ± 27 and $599 \pm 24 \mu\text{m}$, respectively ($P < 0.001$). Using the mixed effects model where eye,

time and method of measurement were controlled, treatment with 5% NaCl ointment ($P < 0.001$) resulted in a 12 μm predicted mean decrease in corneal thickness from pre-treatment values (Figure 3A) and corneal thickness values significantly differed between eyes treated with 5% NaCl versus AT ointment ($P < 0.001$; Figure 3A). There was no significant difference in corneal thickness between values obtained prior to and following treatment with AT ointment ($P = 0.82$; Figure 3A) though a location and treatment interaction was identified with this model. Thus, CCT was also assessed using mixed effects modeling again controlling for eye, time and method of measurement and CCT was significantly lower by 19 μm between eyes treated with 5% NaCl versus AT ointment ($P < 0.001$; Figure 3B). However, CCT was significantly less following treatment with 5% NaCl or AT ointment in comparison to baseline values ($P < 0.001$; Figure 3B).

IVCM

On day 10, epithelial cell density significantly increased following the final treatment with 5% NaCl and AT ointment versus baseline values ($P = 0.003$ and 0.005 , respectively; Table 1). By contrast, keratocyte cell density in the anterior and posterior stroma and endothelial cell density did not significantly differ following treatment with 5% NaCl or AT ointment in comparison to pre-treatment measurements ($P > 0.05$, Table 1). Irrespective of treatment, keratocyte cell density was significantly greater in the anterior versus posterior stroma ($P = 0.001$, Table 1).

Discussion

The present study demonstrated a 2–4% decrease in corneal thickness following topical treatment with 5% NaCl ointment in normal dogs. These results contrast with a study in normal rabbits whereby 5% NaCl in a patented water-soluble polymer (Adapt^R) resulted in an 8–10% decrease in corneal thickness.⁶ Furthermore, 5% NaCl in distilled water did not significantly alter corneal thickness while the Adapt^R vehicle alone significantly reduced corneal thickness by 4%.⁶ Thus, the delivery vehicle for 5% NaCl likely plays a role in maximizing the efficacy of this hyperosmotic at decreasing corneal thickness. These data in aggregate suggest that 5% NaCl ophthalmic ointment may be more efficacious than solution at decreasing corneal thickness in dogs although investigations of the 5% NaCl ophthalmic solution are warranted. These data also suggest that 5% NaCl is more efficacious at decreasing corneal thickness in rabbits versus dogs and possibly due to differences in corneal structure and cellular function between these two species. For example, median central corneal thickness is reported to be approximately 380 μm in rabbits and is thus about 2/3 as thick as the canine central cornea.^{26,27} This may result in increased penetration or improved efficacy of 5% NaCl in rabbits versus dogs due to differences in mass transport properties or intrinsic hygroscopic attributes of the stromal elements.^{26,27} In addition, marked differences in collagen organization and orientation between these two mammalian species may impact the efficacy of 5% NaCl.^{28,29}

In addition to decreasing thickness of normal corneas in dogs and rabbits, 5% NaCl is also effective at reducing corneal thickness in human patients with corneal edema. In 1971, Luxenberg and Green evaluated the efficacy of topically applied corn syrup, sulphacetamide,

and 5% NaCl in combination with one of several vehicles including petrolatum, glycerin, gum cellulose, and methylcellulose which were each applied four times daily for two weeks.⁵ This study demonstrated that 5% NaCl ointment reduced corneal thickness up to 20% for 3–4 h following treatment and maintained a 12% reduction in corneal thickness for up to 7 h after administration.⁵ Of all agents tested, 5% NaCl had the greatest efficacy and longest duration and was well tolerated by the majority of human patients with corneal edema.⁵ Disease severity appears to play an important role in the efficacy of 5% NaCl in human patients. Knezovic and coauthors demonstrated that 5% NaCl solution administered 4 times daily improved visual acuity and decreased corneal thickness to a greater degree in patients with early versus advanced bullous keratopathy.⁸ Improvement of visual acuity and corneal edema was also demonstrated in human patients with bullous keratopathy when treated with the combination of a hydrophilic contact lens and hypertonic saline.¹² Persistent corneal edema due to endothelial dystrophy or degeneration is a relatively common problem in dogs. Thus, the efficacy of 5% NaCl in dogs with edematous corneas is an important subject of future investigation, and it is likely that 5% NaCl would result in a greater decrease in corneal thickness in dogs with edematous versus normal corneas. The area of the cornea affected with edema and corneal thickness should be quantified when evaluating the efficacy of 5% NaCl in canine patients given that its efficacy changes with disease severity in human bullous keratopathy patients.⁸ In addition, a treatment group that includes a hydrophilic contact lens should be considered for inclusion in future studies to determine if a similar benefit in canine patients with corneal edema could be obtained.

Numerous other osmotic agents including glycerin, hypertonic glucose and sulphacetamide have been evaluated but these treatments were either too irritating or their benefit too transient to be clinically applicable. The first treatment proposed for corneal edema was 40% glucose which was first introduced in 1940.³⁰ Karo syrup containing glucose, dextrose, and salt, was difficult to work with and did not display advantages over hypertonic saline.⁵ Topical glycerin resulted in significantly greater corneal thinning compared with either a colloidal dextran polysaccharide or 5% sodium chloride solutions;^{31,32} however, it was so irritating that it required topical anesthetic prior to its use and few patients tolerated it long-term. By contrast, colloidal hyperosmotic solutions were found to be well tolerated and effective in treating recurrent corneal erosions.¹¹ Thus, it is possible that other osmotic agents may be more efficacious in dogs versus humans and investigation of the safety and efficacy of topical glycerin, hypertonic glucose, colloidal hyperosmotic solutions or sulphacetamide should be evaluated in dogs with normal and edematous corneas.

In dogs, evaluation of the efficacy of osmotic agents relies primarily on objective measurements of corneal thickness rather than more subjective parameters such as visual acuity that are more difficult to assess than in humans. In the present study, USP significantly overestimated CCT compared with FD-OCT by 19 μ m and these results are consistent with previous studies in both canine and human patients.^{18,19,33,34} Ultrasonic pachymetry is objective, highly repeatable, and simple to use in the clinical setting eliminating the need for sedation in most situations. Disadvantages of this method include the requirement of corneal contact when acquiring measurements, the variability of the speed of sound utilized by various machines, and user variability.^{18,19,33,34} The necessity of probe contact requires instillation of topical anesthesia, increased likelihood of patient

discomfort, and risk of microbial contamination and epithelial alterations.^{35,36} To determine thickness, velocity derived from the human cornea is assumed, which may contribute to inaccuracies and likely contributed to the overestimation of thickness observed with USP versus FD-OCT in the present study.^{19,33}

With advancements in technology, use of alternative modalities such as FD-OCT are increasing in veterinary ophthalmology particularly for patients with corneal disease.^{13–16} In FD-OCT, corneal thickness measurements are obtained using non-invasive, high resolution, cross-sectional imaging of the cornea but this technique is slower to perform, requires greater expertise, and requires sedation for some canine patients. With this noncontact method, measurements rely on the creation of signals at interfaces as a result of reflection of infrared waves from the anterior and posterior corneal surfaces.^{16,37} The uncertainty of the true index of refraction of infrared radiation in the cornea creates a source of error in calculating the CCT with OCT.³⁸ While a statistically significant difference exists between the FD-OCT and USP readings in the present study, the difference is unlikely to make a difference in the clinical interpretation of data and both instruments can be used reliably to measure corneal thickness in dogs.

In the present study, use of two different USP techniques, Accupach and Pachette, did not significantly differ and a strong correlation was observed. Previous studies have shown good repeatability between various ultrasonic pachymeters^{39–42} while others have shown clinically important variability.^{43–47} Placement of the probe less than perpendicular to the cornea or in different positions on the cornea likely affects the precision of ultrasonic pachymeters.⁴⁸ The Accupach and Pachette use the same corneal velocity setting of 1640 m/sec but utilize 65 and 20 mHz probes, respectively. The Accupach collects individual readings of which 3 were averaged in the present study. By contrast, the Pachette acquired 25 consecutive readings at a single placement position which decreases the chance of a spurious low reading.⁴⁸ The indentation of the cornea by each pachymeter may have varied between the two pachymeters as a longer contact time was required for the Pachette although indentation does not markedly affect ultrasound measures of corneal thickness.⁴⁹

The multifocal superficial punctate fluorescein stain retention noted at the end of each day and the superficial corneal ulcer in one dog was suspected to be related to the frequent use of topical proparacaine needed to acquire the USP readings. While it is possible that this stain pattern was related to frequent acquisition of pachymetry data points, we noted that it was diffuse and not limited to the perilimbal superior, inferior, nasal, temporal, and central locations in contact with the ultrasonic probe. Toxic effects of topical anesthetic are suggested to be direct as even a single dose of topical anesthetic may cause mild punctate epithelial keratopathy.⁵⁰ Studies have shown that anesthetics interrupt migration of corneal epithelial cells⁵¹ and directly damage the epithelial cell organelles, desmosomes, and cytoskeletal elements by altering cellular metabolism and function.^{52–54} Frequency of administration also plays a critical role in the toxicity of topical proparacaine as a single dose resulted in a decrease in the number of microvilli and microplicae but multiple applications resulted in increased epithelial cell desquamation and disruption to the plasma membrane and cytoplasm.⁵¹ Topical anesthetics also produce a decrease in keratocyte viability via a direct cytotoxic effect,⁵⁵ and impairment of keratocyte and endothelial cell

metabolism has also been associated with topical anesthetic use.⁵⁵ The preservatives in topical anesthetics have also been suggested to be toxic to corneal cells.^{52,56–58} Thus, corneal toxicity of topical anesthetics should be considered when determining the frequency of USP readings collected for prospective studies where evaluation of corneal thickness is required.

Increased epithelial cell density in the central cornea as measured by IVCN and decreased CCT was noted with both 5% NaCl and AT versus baseline values. In both humans and dogs, basal epithelial cell density is higher than superficial epithelial cell density.^{22,59} Thus, it is possible that premature desquamation of epithelial cells occurred as a result of the chronic and frequent administration of topical proparacaine and/or preservatives found in it.^{51–58,60–62} A similar loss of superficial epithelial cells and reduction in surface cell size has been seen with mild ocular irritants.⁶¹ Benzalkonium chloride, a preservative commonly found in topical ophthalmic preparations, resulted in superficial epithelial cell desquamation and clinical corneal toxicity.⁶² The proparacaine used in this study contained 0.01% benzalkonium chloride while the AT and 5% NaCl ointments used in this study were preservative-free. This epithelial cell desquamation coupled with mild desiccation in the central cornea from the repeated USP measurements may have accounted for the significant decrease in CCT observed with the AT treatment in comparison to baseline.

In conclusion, this study provides the first objective data that 5% NaCl produces a mild, though significant decrease in corneal thickness in normal dogs. Future studies assessing the effect of hypertonic saline in dogs with corneal edema are warranted. Based on the current study and previous studies in other species, we would expect a greater effect to be observed with 5% NaCl in edematous versus normal canine corneas.

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Figure 1.

Multifocal punctate superficial corneal erosions in the left eye a 2-year-old intact female Beagle during day 3 of the study receiving AT ointment prior to (A) and following fluorescein stain (B). The image in (A) was obtained with a Nikon D10 camera attached to a Nikon autofocus micro Nikkor 105 mm (1:2.8 D) lens and a Nikon macro speedlight SB-29s ring flash while (B) was obtained after topical fluorescein stain application with a Nikon D300 SLR digital camera with BLUE-AWB filters placed over the twin lights (Nikon wireless remote speed light SB-R200) and a yellow barrier filter over the lens (HOYA HMC 62mm Y [k2] filter).

Figure 2.

Least square linear regression plot demonstrating corneal thickness measurements obtain via USP with the Pachette and Accupach. Corneal thickness measurements exhibited a high correlation ($R^2 = 0.960$) and did not significantly differ ($P = 0.593$) between the two pachymeters. In the present study, 922 measurements in both eyes of 5 dogs were taken simultaneously allowing these two instruments to be compared.

Figure 3.

Predicted mean $\pm$ standard error corneal thickness (**A**) and CCT (**B**) prior to and following treatment with 5% NaCl or artificial tears (AT) ointment at least four times daily for 9 days in 5 intact female Beagles as analyzed using mixed effects linear regression. Treatment with 5% NaCl ointment significantly decreased ($P < 0.001$) corneal thickness in comparison to baseline and AT ointment, but there was no significant difference in corneal thickness between predicted values obtained pre-treatment and following treatment with AT ointment ($P = 0.82$, **A**). By contrast, treatment with 5% NaCl or AT ointment significantly decreased ($P < 0.001$) CCT in comparison to baseline (**B**). However, treatment with 5% NaCl versus AT ointment resulted in a significantly greater reduction in CCT ($P < 0.001$, **B**).

Table 1

Mean $\pm$ SD density of corneal cells following in vivo confocal microscopy of the central cornea of 5 intact female beagles prior to and following treatment with 5% NaCl or artificial tear (AT) ophthalmic ointment at least four times daily for 9 days.

	Cell Density (cells/mm ²)		
	Before treatment	5% NaCl	AT
Epithelium	531 $\pm$ 53	577 $\pm$ 43 *	567 $\pm$ 15 *
Anterior Stroma	753 $\pm$ 29	768 $\pm$ 28	779 $\pm$ 39
Posterior Stroma	745 $\pm$ 33	713 $\pm$ 29	722 $\pm$ 50
Endothelium	2367 $\pm$ 169	2436 $\pm$ 189	2513 $\pm$ 237

* Significantly differed in comparison to before treatment ($P = 0.005$).