Comparison of efficacy and duration of effect on corneal sensitivity among anesthetic agents following ocular administration in clinically normal horses

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Objective—To compare efficacy and duration of effect on corneal sensitivity of 0.5% proparacaine hydrochloride, 0.5% bupivacaine hydrochloride, 2% lidocaine hydrochloride, and 2% mepivacaine hydrochloride solutions following ocular administration in clinically normal horses.

Animals—68 clinically normal horses.

Procedures—60 horses were assigned to receive 1 anesthetic agent in 1 eye. For each of another 8 horses, 1 eye was treated with each of the anesthetic agents in random order with a 1-week washout period between treatments. Corneal sensitivity was assessed via corneal touch threshold (CTT) measurements obtained with a Cochet-Bonnet aesthesiometer before and at 1 minute, at 5-minute intervals from 5 to 60 minutes, and at 10-minute intervals from 60 to 90 minutes after application of 0.2 mL of anesthetic agent. General linear mixed models were fitted to the CTT data from each of the 2 experimental groups to assess the effects of the anesthetic agents over time, accounting for repeated observations within individual horses.

Results—Corneal sensitivity decreased immediately following topical application of each anesthetic agent; effects persisted for 35 minutes for proparacaine and mepivacaine treatments, 45 minutes for lidocaine treatment, and 60 minutes for bupivacaine treatment. Maximal CTT reduction was achieved following application of bupivacaine or proparacaine solution, whereas mepivacaine solution was least effective.

Conclusions and Clinical Relevance—Ocular application of each evaluated anesthetic agent reduced corneal sensitivity in horses; although 0.5% proparacaine or 2% lidocaine solution appeared to induce adequate short-duration corneal anesthesia, use of 0.5% bupivacaine solution may be more appropriate for procedures requiring longer periods of corneal anesthesia. (Am J Vet Res 2013;74:459–464)

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Abbreviation

CTT  Corneal touch threshold

cell layers and anterior stroma are richly innervated, with unmyelinated nerve endings present in the epithelium. The density and distribution of corneal nerves have been extensively studied in humans, dogs, cats, and rabbits but have not been determined to date in horses, to our knowledge. Since then, many aesthesiometers have been developed, including the Cochet-Bonnet corneal aesthesiometer. The Cochet-Bonnet corneal aesthesiometer has become the most commonly used aesthesiometer in both clinical and research settings. This device uses a 0.12-mm-diameter nylon monofilament of variable exposed lengths from 6.0 to 0.5 cm, which, when applied to the corneal surface, correspond to pressures of 11 to 200 mg/0.0113 mm².
respectively. A longer filament length applies less pressure to the corneal surface, whereas a shorter filament is more rigid and applies more pressure. Limitations to its use include subjective interpretation of the response, subject apprehension, a lack of standardization of the filament deflection pressure, and variations in technique.

Corneal touch threshold is the minimal amount of corneal stimulation that results in a blink reflex and is measured by use of a corneal aesthesiometer. The CTT has been studied as a measure of corneal sensitivity in horses of various ages and breeds, and its mean value has been reported to range from 2.12 ± 0.62 cm to 5.01 ± 0.61 cm as determined by use of the Cochet-Bonnet corneal aesthesiometer. Previous studies in horses have revealed that the central portion of cornea is the most sensitive, followed in order of decreasing sensitivity by the nasal, temporal, ventral, and dorsal regions. It is presumed that these location-related differences in corneal sensitivity reflect the relative abundance in nerve fiber density at these locations because the central portion of the cornea in other animals has the greatest corneal nerve fiber density.

Topical corneal anesthesia is frequently used in humans and other animals for a variety of diagnostic procedures, including measurement of intraocular pressure, collection of cytologic samples from the cornea or conjunctiva, examination of eyes with painful corneal diseases, paracentesis of the anterior chamber, and removal of ocular foreign bodies. Various anesthetic agents are used for corneal anesthesia in veterinary medicine, one of the most commonly used and studied anesthetic agents is 0.5% proparacaine hydrochloride ophthalmic solution. This drug formulation has a rapid onset of action (< 5 minutes in most species) with a variable duration of effect (durations of 25 minutes in horses and cats and 45 minutes in dogs). The manufacturer recommends keeping the solution refrigerated when not in use, and the drug’s efficacy decreases if the solution is not maintained accordingly. The storage requirement for proparacaine ophthalmic solution is difficult to maintain in certain clinical situations and when traveling to evaluate patients in the field. Formulations of bupivacaine, lidocaine, tetracaine, and mepivacaine, which do not require refrigeration, have been investigated for topical ophthalmic use in humans and various other species, but the comparative efficacy of these local anesthetic agents in equine eyes has not been evaluated to our knowledge.

The purpose of the study reported here was to compare the efficacy and duration of effect on corneal sensitivity of 0.5% proparacaine hydrochloride, 0.5% bupivacaine hydrochloride, 2% lidocaine hydrochloride, and 2% mepivacaine hydrochloride solutions following ocular administration in clinically normal horses. Corneal sensitivity was determined on the basis of CTT measurements obtained via corneal aesthesiometry. The anesthetic agents evaluated were chosen because of their common and effective use in humans as well as ready availability to veterinary practitioners.

Materials and Methods

Animals—The study involved 60 Quarter Horses from the Kansas State University Animal Science and Industry Horse Unit and 8 privately owned horses of various breeds. Among the 68 horses, there were 24 geldings and 44 mares ranging in age from 2 to 20 years (mean, 6.3 years). Breeds represented included Quarter Horses (n = 64), Arabian or Arabian-cross (2), and American Saddlebred (1). All horses underwent an ophthalmic examination consisting of slit-lamp biomicroscopy examination, Schirmer tear test, and rebound tonometry. Only animals deemed to be clinically normal and for which Schirmer tear test results were > 10 mm/min were included. The experimental procedures were performed with gentle manual restraint of the horses without the use of sedation. This study was approved by the Institutional Animal Care and Use Committee of Kansas State University. Written consent from owners or agents was obtained prior to study participation.

Measurements—A Cochet-Bonnet aesthesiometer was used to measure CTT in 1 randomly selected eye of each horse (32 right eyes and 36 left eyes). The aesthesiometer contained a 0.12-mm-diameter nylon filament of adjustable length (6.0 to 0.5 cm) that was gently applied perpendicular to the central portion of the cornea to determine corneal sensitivity. Pressure was increased until a minimal deflection of the filament was noted. Aesthesiometer filament readings can be converted to applied force measurements via a conversion chart provided by the manufacturer. Readings are then displayed as either grams per square millimeter or milligrams per square millimeter (S = 0.0113 mm² of sectional area of the filament).

On each occasion, a baseline CTT was obtained for the selected eye of all horses prior to administration of the anesthetic agent. The baseline CTT was determined by applying the filament to the central portion of the cornea at maximal length (6 cm) and monitoring for a blink response. If no response was noted, the filament was decreased in length by 0.5-cm increments and reapplied until a blink reflex occurred in response to at least 3 of 5 stimulations. The length of the filament that stimulated a reproducible blink response was recorded as the CTT measurement (in centimeters). Each horse was then administered one of the drug solutions topically in that same eye, and measurements were obtained at intervals during a period of 90 minutes after application. The CTT was recorded as 0 cm if no response was noted with application of the maximal stimulus (ie, shortest filament length, 0.5 cm), indicating complete corneal anesthesia. The same investigator (JDP) performed all measurements, thereby minimizing a confounding variable of interobserver variance. Care was taken to avoid manipulation of eyelids and stimulation of vibrissae. Ambient temperature was recorded for each day on which testing was performed.

Treatment—The 68 horses were assigned to 1 of 2 groups, and each group participated in a different experimental design. Horses in group 1 were randomly assigned to receive ocular treatment with an ophthalmic solution of 0.5% proparacaine hydrochloride or injectable solutions of 0.5% bupivacaine hydrochloride, 2% lidocaine hydrochloride, or 2% mepivacaine hydrochloride. For each horse, the eye to be treated and tested was selected at random. Horses in group 2 were assigned to receive all 4 treatments in a...
replicated Latin square design with a 1-week washout period between treatments. For these 8 horses, the eye to be treated and tested was selected at random prior to application of the first drug; this eye was also used for the other 3 drug treatment evaluations.

After determination of baseline CTT for each horse prior to administration of the anesthetic agent, 0.2 mL of the selected drug solution was drawn into a 1-mL syringe via a 27-gauge needle. The needle was broken off at the hub, and the drug solution was gently sprayed onto the dorsal corneal surface. The same bottle of each drug solution was used throughout the study period and stored according to the manufacturer's guidelines. The time of topical administration of the anesthetic agent was designated as 0 minutes; following administration, the treated eyes were monitored for any adverse reaction to the drug solution. Corneal touch threshold was then measured at 1 minute, at 5-minute intervals from 5 to 60 minutes, and at 10-minute intervals from 60 to 90 minutes after application of the drug solution. Each measurement after the application of the drug solution under evaluation was initiated at a length 1.5 cm longer than that used for the preceding stimulation and decreased in 0.5-cm increments until a consistent blink response was detected in response to 3 of 5 stimulations. After completion of the final measurement, corneas of both eyes were stained with fluorescein and examined with a slit lamp to ensure that the corneal epithelium was intact.

Data analysis—Data from each experimental group were analyzed separately to reflect the different underlying experimental designs. In both experimental groups, a general linear mixed model was fitted to the response CTTs assuming a Gaussian data distribution. The models included the fixed effects of drug treatment (0.5% proparacaine, 0.5% bupivacaine, 2% lidocaine, or 2% mepivacaine solution), time (0 to 90 minutes), and their 2-way drug-time interaction. The fixed effect of sex as well as the covariates age and ambient temperature were evaluated for inclusion in the model on the basis of their $P$ values. In the statistical model fitted to data from group 1 (completely randomized design experiment), a first-order antidependence structure was used to model the residual variance-covariance matrix to accommodate heterogeneous residual variances and to account for repeated observations on a given horse over time. In the statistical model fitted to data from group 2 (replicated Latin square experiment), the random blocking factors of horse and period were included. A spatial power variance-covariance structure was fitted to the residuals to account for repeated observations on a given horse-period-drug combination over time. All variance-covariance structures were selected on the basis of a model fit via the Bayesian information criterion.

The Kenward and Roger approach was used for computing degrees of freedom and estimating SEs. The models were fitted with statistical software via a generalized linear mixed models procedure. Model assumptions were evaluated via Studentized residual plots, and assumptions were considered to be appropriately met. Pairwise comparisons were conducted via Bonferroni adjustment to avoid inflation of type I error rate. Values of $P < 0.05$ were considered significant.

Results

Mean baseline CTT values (obtained prior to administration of the anesthetic agent) did not differ among treatments. For each of the 4 drugs evaluated in the present study, the onset of action was rapid; the maximal effect was evident within 5 minutes after administration. Corneal touch threshold values for each treated eye returned to baseline CTT value within 90 minutes after application, regardless of the specific anesthetic agent applied. For both experimental groups, there was evidence of a significant ($P < 0.001$) drug-time interaction on CTT values, indicating that the dynamics of corneal sensitivity over time differed among the anesthetic agents. The estimated least squares mean CTT values for each drug solution over time for the completely randomized design and replicated Latin square design experimental groups were illustrated (Figures 1 and 2). No adverse effects after ocular application of any drug solution were detected in any horse during the period of the study. After completion of the final measurement, fluorescein staining and slit-lamp examination revealed that the corneal epithelium of both eyes in all horses was intact.

There was no evidence of any association between ambient temperatures, as recorded on testing days, and CTT measurements. Also, there was no evidence of an effect of sex on CTT values in the present study. However, a significant ($P = 0.009$) positive effect of age on CTT value was apparent, wherein each 1-year increase in age was associated with a mean ± SE estimated increase of 0.02 ± 0.01 cm of CTT regardless of treatment.

Group 1 (completely randomized design experimental group)—For horses in group 1, least squares
Comparison of differences in CTT over time relative to the effect of the anesthetic agents was assessed by comparing differences in CTT over time with respect to baseline CTT value for each drug solution. Corneal sensitivity was significantly decreased relative to baseline value at all time points up to 35 minutes after application for proparacaine solution (all $P < 0.001$), at all time points up to 40 minutes for mepivacaine solution (all $P < 0.004$), and at all time points up to 55 minutes after application for lidocaine solution (all $P < 0.04$), and at all time points up to 60 minutes after application for bupivacaine solution ($P < 0.02$; Figure 2).

**Discussion**

Results of the present study indicated that ocular application of each of the 0.5% proparacaine, 0.5% bupivacaine, 2% lidocaine, and 2% mepivacaine solutions evaluated decreased corneal sensitivity in clinically normal horses. The mean baseline corneal sensitivity (determined via CTT measurement) did not vary significantly among drug solution treatments within a given experimental group (the completely randomized design experimental group or the replicated Latin square experimental group); baseline CTT values ranged from 2.9 to 3.9 cm, which is in accordance with results of previous studies$^{7-10,22}$ in horses that were evaluated by use of the same type of aesthesiometer. After treatment administration, CTT values differed significantly from the respective baseline value for some time, depending on drug solution; among the drug solutions, significant differences in CTT values were detected at various time points after application. For each drug solution evaluated, corneal desensitization was evident at 1 minute after application and the maximal drug effect was detected at 5 minutes after application. The duration of effect on corneal sensitivity varied among drug solutions, with bupivacaine solution providing the longest-acting effect.

In contrast to findings of a recent study,$^{6}$ the proparacaine solution used in the present study decreased corneal sensitivity to a level approaching complete anesthesia, and CTT values remained significantly decreased relative to baseline value at all time points up to 35 minutes after application. The difference in corneal anesthetic effects of proparacaine solution between the 2 studies could be breed related because the corneal anesthetic effects of proparacaine solution reported in the present study, a formal comparison of CTT values among breeds could not be conducted. The duration of corneal anesthetic effect of the drug solutions in the present study was also longer than durations reported for horses and cats$^{14,15}$ but shorter than that reported for dogs. However, it may be inappropriate to compare duration of the anesthetic effect among species, considering that a larger volume of drug solution

mean ± SE baseline CTT results were not significantly different among treatments and ranged from 2.96 ± 0.14 cm to 3.04 ± 0.14 cm. Relative to baseline CTT value, corneal sensitivity decreased considerably at 1 minute after application of each of the 4 drug solutions ($P < 0.001$), and a minimum CTT value was recorded at 5 minutes after application. The minimum estimated CTT values at 5 minutes after application were 0.01 ± 0.06 cm, 0.00 ± 0.06 cm, 0.03 ± 0.06 cm, and 0.4 ± 0.06 cm for proparacaine, bupivacaine, lidocaine, and mepivacaine solutions, respectively. The duration of effect of the anesthetic agents was assessed by comparison of differences in CTT over time relative to the baseline CTT value for each drug solution. Corneal sensitivity was significantly decreased relative to baseline value at all time points up to 35 minutes after application for the proparacaine (all $P < 0.03$) or mepivacaine (all $P < 0.001$) solution, at all time points up to 45 minutes after application for the lidocaine solution (all $P < 0.001$), and at all time points up to 60 minutes after application for the bupivacaine solution ($P < 0.03$; Figure 1).

**Group 2 (replicated Latin square experimental group)**—For horses in group 2, least squares mean ± SE baseline CTT results were not significantly different among treatments and ranged from 3.65 ± 0.21 cm to 3.88 ± 0.21 cm. Relative to baseline CTT value, corneal sensitivity had decreased rapidly at 1 minute after application of each of the 4 drug solutions ($P < 0.001$), and a minimum CTT value was recorded at 5 minutes after application. The minimum estimated CTT for each drug solution at 5 minutes after application was 0.03 ± 0.21 cm, 0.02 ± 0.21 cm, 0.25 ± 0.22 cm, and 0.82 ± 0.21 cm for proparacaine, bupivacaine, lidocaine, and mepivacaine solutions, respectively. The duration of effect of the anesthetic agents was assessed by comparing differences in CTT over time relative to the baseline CTT value for each drug solution. Corneal sensitivity was significantly decreased relative to baseline value at all time points up to 35 minutes after application for proparacaine solution (all $P < 0.001$), at all time points up to 40 minutes for mepivacaine solution (all $P < 0.004$), and at all time points up to 55 minutes after application for lidocaine solution (all $P < 0.04$), and at all time points up to 60 minutes after application for bupivacaine solution ($P < 0.02$; Figure 2).
tion was applied to the corneal surface of horses in the present study, compared with the single drop of solution applied to the corneal surface of the cats and dogs in the other experiments.13,16

The application of 2% lidocaine solution to the corneal surface appeared to provide efficacy and duration of corneal anesthesia comparable to that provided by application of 0.5% proparacaine solution in clinically normal horses. Lidocaine solution has been used empirically in clinical practice to desensitize corneas of horses, but to our knowledge, this is the first reported evaluation of its use. The injectable formulation of lidocaine used in the present study is more commonly used in veterinary medicine for local nerve blockade and treatment of ventricular arrhythmias. In humans’ eyes, lidocaine can be used for many diagnostic and therapeutic procedures performed on the cornea and anterior segment, including phacoemulsification.19,20,24,25

Given the lack of refrigeration requirements and activity that is apparently comparable to that of proparacaine ophthalmic solution, lidocaine solution is a potential alternative anesthetic agent for corneal desensitization in horses. Topical use of an ophthalmic lidocaine gel formulation was not evaluated in the present study, so the effects of such a preparation in horses cannot be described.

In humans, bupivacaine solution is used for topical, subconjunctival, retrobulbar, and intracameral anesthesia with adequate effect.18,20,26,27 To our knowledge, there have been no reports of studies evaluating topical application of the injectable formulation of bupivacaine for corneal anesthesia in horses. Prolonged corneal anesthesia, relative to the effects achieved via application of the other drug solutions evaluated, was noted after ocular application of 0.5% bupivacaine solution in the horses of the present study. This finding is in accordance with the prolonged effect noted when bupivacaine is used in other regions of the body.26,27 The duration of action of bupivacaine is reported to be related to its high degree of protein binding and lipid solubility.18,20,27 This duration of effect could be beneficial when performing procedures for which a longer duration of corneal anesthesia is necessary.

Mepivacaine is most commonly used in veterinary medicine to provide local nerve blockades. This drug is also used topically and intracameral in humans during cataract surgery or other ocular procedures and provides adequate desensitization of the cornea.30,31 Although there was a reduction in corneal sensitivity following ocular application of mepivacaine solution in the horses in the present study, the anesthetic effect of this treatment was not as notable as that provided by application of each of the other anesthetic agents. The comparatively decreased efficacy of mepivacaine is most likely related to its poor corneal permeability, as shown in rabbits.32 On the basis of the findings of the present study, we do not recommend topical application of 2% mepivacaine solution for ophthalmic anesthesia in horses.

Amide- or ester-based anesthetic drugs, such as those investigated in the present study, exert their activity by penetrating the corneal surface and inducing a reversible nerve conduction block following topical application to the cornea. The blockade occurs in the regions of the sub-basal nerve plexus, where the nerves are only loosely protected by Schwann cells.18,27 This blockade is thought to develop by decreasing the permeability of the nerve cell membrane to sodium and other ions, thereby inhibiting depolarization and halting propagation of pain sensations.26,32 The rapid onset of action of the drugs used in the present study is likely due to their high lipid solubility, which allows drug molecules to penetrate cell membranes and be rapidly presented to the target receptors.26,27,32 The pH of the drug formulation also plays a role in the degree of block provided because only the nonionized drug particles are available to bind to the receptors. The closer the pH is to the pKa for a selected drug, the greater the number of nonionized particles that are present.

Previous reports1 have proposed that alterations in corneal sensitivity in humans are associated with hormonal status, gender, age, ambient temperature, humidity, and ambient lighting. In the present study, we attempted to minimize these factors by having the same investigator (JDP) perform all testing, by use of the same materials, and by testing each horse in a similar environment. The study methods appeared to be adequate, considering that all CTT values in all horses returned to their respective baseline value by the end of the manipulations; furthermore, horses in experimental group 2 had numerically similar baseline CTT values prior to each treatment. In the present study, we were not able to detect differences in CTT as a function of ambient temperature or sex. Among the study horses, there was a significant difference in CTT values with increasing age, as has been found in humans.3 However, the estimated magnitude of the association between age and decreased corneal sensitivity, although significant, is not likely to be clinically relevant.

Potential limitations of the present study were that the investigators were not masked to the drug solution applied and toxic effects of the treatments on the corneal epithelium were not evaluated. An attempt to prevent potential bias was made by randomly assigning group 1 horses to drug treatments and by enrolling group 2 horses in an experiment designed to evaluate the effect of each drug within the same individual. No overt adverse clinical effects were detected after application of the drug solutions at any time point, but without a microscopic examination of the corneal epithelial cells, the safety of topical application of the evaluated anesthetic agents cannot be definitively ascertained. However, in humans, these drugs are viewed as generally safe when used by ophthalmologists in a controlled clinical setting.31

Lastly, the present study was performed on clinically normal horses, so it is not known how these drug solutions would affect horses with corneal disease. A previous study34 revealed that in diseased states, the pH of the corneal surface is lower than that in healthy eyes, leading to an increase in blood flow through corneal blood vessels and reduction in the efficacy of the agents through dilution. Future studies need to be conducted to determine the efficacy and duration of effect of the evaluated drug solutions in horses with various corneal diseases.
On the basis of the results of the present study, ocular application of 0.2 mL of 0.5% proparacaine ophthalmic solution or 2% lidocaine injectable formulation appeared to provide a marked reduction in corneal sensitivity in clinically normal horses, which should be of benefit for short-duration procedures. Ocular application of 0.5% bupivacaine injectable formulation may be useful for procedures in which a longer duration of decreased corneal sensitivity is needed. Although ocular application of the 2% mepivacaine injectable formulation significantly decreased corneal sensitivity, compared with the pretreatment baseline CTT value, the use of this drug solution is not recommended because the magnitude of corneal desensitization was less than that achieved via administration of the proparacaine, lidocaine, or bupivacaine solution. Regardless of the anesthetic agent applied to the eyes of horses, it is important to provide lubrication to the corneal surface during periods of corneal anesthesia because lacrimation stimulation and the blink reflex may be temporarily reduced.

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