Effects of action of proparacaine and tetracaine topical ophthalmic formulations on corneal sensitivity in horses

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**Objective**—To compare the corneal anesthetic effects and duration of action of 2 ophthalmic anesthetic agents in horses.

**Design**—Prospective, randomized masked crossover study.

**Animals**—8 clinically normal adult horses.

**Procedures**—Corneal sensitivity was determined by measuring each eye’s corneal touch threshold (CTT) with a Cochet-Bonnet esthesiometer. Each eye’s baseline CTT was recorded prior to anesthetic instillation at 0 minutes and every 10 minutes thereafter for 60 minutes. Each eye was randomly assigned to receive 2 of 4 treatments: 0.5% aqueous proparacaine ophthalmic solution (aqueous proparacaine; 8 eyes); 0.5% aqueous tetracaine ophthalmic solution (aqueous tetracaine; 8 eyes); 0.5% viscous tetracaine ophthalmic solution (viscous tetracaine; 8 eyes); and saline (0.9% NaCl) eyewash solution (8 eyes) as a negative control. There was a 48-hour washout period. Every horse received all treatments.

**Results**—Median baseline CTT of eyes was 4.5 cm (range, 0.5 to 6 cm). Median CTT for saline solution–treated eyes never differed significantly from baseline. The maximum anesthetic effect with the other 3 treatments occurred at 10 minutes. Median CTT at 10 minutes was 0.5 cm (range, 0 to 2.5 cm) with aqueous proparacaine treatment, 0.25 cm (range, 0 to 2.0 cm) with aqueous tetracaine treatment, and 0 cm (range, 0 to 0.5 cm) with viscous tetracaine treatment. Maximum anesthetic duration was 20 minutes with aqueous proparacaine and aqueous tetracaine treatments and 30 minutes with viscous tetracaine treatments.

**Conclusions and Clinical Relevance**—Treatment of eyes with viscous tetracaine resulted in the greatest decrease in CTT and the longest duration of action, compared with treatment with aqueous proparacaine or aqueous tetracaine. (J Am Vet Med Assoc 2012;241:1645–1649)

Abbreviation

| CTT | Corneal touch threshold |

After the application of 0.2 mL of proparacaine. However, Kalf et al. demonstrated that proparacaine never completely desensitizes the equine cornea. Recently, another topical anesthetic agent, tetracaine, has been shown to completely desensitize the equine cornea. Although pain and chemosis have been reported after topical application of tetracaine to canine eyes, tetracaine was reported to be well tolerated in horses. The maximal duration of effect was prolonged by increasing the tetracaine concentration from 0.5% to 1% or by instilling 2 drops 1 minute apart. A viscous formulation of tetracaine is also available, but its ability to desensitize the equine cornea has not been examined. Theoretically, the increased viscosity should increase corneal contact time and thereby result in a superior degree of anesthesia and prolonged duration.

To our knowledge, no studies have been reported that directly compare the efficacy of proparacaine with that of tetracaine on equine corneas. The objective of the study reported here was to compare the efficacy and duration of action of proparacaine and tetracaine on corneal sensitivity in horses. Two formulations of tetracaine, a 0.5% aqueous solution and a 0.5% viscous gel, were used.
Materials and Methods

Animals—Eight healthy adult horses from the Michigan State University College of Veterinary Medicine teaching and research herds were used in the study. Horses were excluded if they were noncooperative, had a history of corneal disease, or had evidence of anterior segment disease as determined by slit-lamp biomicroscopic examination. Horses were excluded if Schirmer tear test values were abnormal (< 15 mm/min) or if either eye retained fluorescein stain. Horses that were receiving NSAIDs the time of the study were excluded because they can influence corneal sensitivity.8 Upper and lower vibrissae were trimmed to 3 cm in length prior to testing to limit inadvertent contact during data collection. The Michigan State University Institutional Animal Use and Care Committee approved the protocol and all procedures in the study reported here.

Treatment—Each eye was randomly assigned to receive 2 of 4 treatments in this randomized, crossover, single-blinded protocol as follows: 0.5% aqueous proparacaine hydrochloride ophthalmic solution (aqueous proparacaine; n = 8 eyes); 0.5% aqueous tetracaine hydrochloride ophthalmic solution (aqueous tetracaine; 8 eyes); 0.5% viscous tetracaine hydrochloride ophthalmic solution9 (viscous tetracaine; 8 eyes); and saline (0.9% NaCl) eyewash solution (8 eyes) as a negative control. To maintain consistent efficacy throughout the study, all solutions were stored in a refrigerator when not in use. Baseline CTT of each eye was recorded prior to instillation of the treatment agent. First, the right and left eyes of each horse were randomly assigned by the roll of a die to separate treatments. No horse received the same treatment in both eyes. Therefore, each horse initially received 2 treatment solutions. The order in which eyes received treatment was randomized by a coin toss. One investigator applied 0.2 mL of the treatment solution onto the cornea. The investigator who applied the treatment solution did not evaluate the CTT because of concerns that the nature of the viscous tetracaine solution would reveal the substance applied.

After at least a 48-hour washout period, each horse received the remaining 2 solutions, again randomly assigned to the right or left eye. In this manner, the right eye of each horse received 2 of the treatment solutions and the left eye of each horse received the other 2 treatment solutions so that each horse received all 4 treatment solutions.

Evaluation of corneal sensitivity—A Cochet-Bonnet esthesiometer was used to determine the CTT.7 The esthesiometer consisted of a 0.12-mm-diameter nylon monofilament that was applied perpendicularly to the corneal surface until a slight bend was detected in the filament. The length of the nylon filament could be varied to the cornea increased from an initial 11 mg/mm2 up to a maximum of 200 mg/mm2. The pressure transmitted by the filament stimulated corneal touch receptors, inducing the corneal blink reflex. The filament length at which the corneal blink reflex was stimulated was recorded as the CTT. The testing was performed on unsedated horses standing in a quiet stall. Minimal head restraint was applied. All CTT recordings were obtained from the central cornea.

The baseline CTT of each eye was determined prior to application of a treatment solution. The first touch was made with the longest thread length of 6 cm. If there was no response to 3 of 5 touches, the filament length was decreased by 0.5-cm increments until the horse had a consistent corneal blink reflex in response to the corneal stimulus. Blinks stimulated by inadvertent contact with eyelashes were excluded. When 3 of 5 touches elicited a corneal blink reflex, the length of the nylon filament was recorded as the CTT. A second investigator was present to instill the treatment solution, restrain the horse, and assist in identification of false-positive reactions resulting from either contact with the eyelashes (palpebral reflex) or blinking prior to contact with the nylon thread (menace response). For each measurement following treatment, the initial length of the nylon filament was matched to that eye’s baseline and decreased in the same manner as the pretreatment CTT values. If a blink was elicited at the previous baseline, the length was increased in 0.5-cm increments until there was no blink response to 3 of 5 touches. The CTT of the eye was recorded as 0 cm if there was no blink response to the shortest filament length of 0.5 cm.

Baseline CTT of each eye was evaluated within 10 minutes prior to application of treatment. Time 0 minutes was recorded as the time at which treatment solution was administered. The CTT was evaluated at 10 minutes after treatment and every 10 minutes thereafter for a period of 60 minutes. All eyes were stained with fluorescein at the completion of testing.

Statistical analysis—Data from CTT measurements were nonnormally distributed. A Friedman test was used to compare CTT measurements among treatment groups at each time point. A Friedman test was also used to compare CTT measurements within each treatment group over time with baseline measurements. A Friedman test was used to compare CTT measurements at each time point for saline solution–treated eyes with the baseline CTT measurements for all other groups of treated eyes. When a value of P < 0.05 was obtained, post hoc analysis with Bonferroni adjustment was performed to determine significant differences between individual variables by evaluation of differences of mean rank. All calculations were performed with statistical software.8 Significance was set at a value of P < 0.05.

Results

Baseline corneal sensitivities among treatments were not significantly (P = 0.787) different. Median CTT of untreated eyes (ie, baseline values) was 4.5 cm (range, 0.5 to 6.0 cm). Median CTT of saline solution–treated eyes never differed significantly (P = 0.102) from its baseline value. Also, median CTT of saline solution–treated eyes at each time point never differed significantly (P = 0.921) from baseline CTT measurements for each of the other treatment groups. At 10 minutes, the maximum anesthetic effect was observed for aqueous proparacaine, aqueous tetracaine, and viscous tetracaine treatments (Figure 1). Median
proparacaine. †Aqueous tetracaine. ‡Viscous tetracaine. §Saline (0.9% NaCl) eyewash control. IIAqueous test solutions resulting in a significantly (each treatment is denoted by an asterisk. At each time point, the significantly different from the maximum anesthetic effect within each eye received 2 treatments and each horse received each of the 4 treatments. The time point at which the CTT was not significantly different from the maximum anesthetic effect within each treatment is denoted by an asterisk: At each time point, the test solutions resulting in a significantly (P < 0.05) different CTT are denoted. †Saline (0.9% NaCl) eyewash control. ‡Aqueous proparacaine. §Aqueous tetracaine. †Viscous tetracaine.

CTT of eyes at 10 minutes was 0.5 cm (range, 0 to 2.5 cm) with aqueous proparacaine treatment, 0.25 cm (range, 0 to 2.0 cm) with aqueous tetracaine treatment, and 0 cm (range, 0 to 0.5 cm) with viscous tetracaine treatment. At 10 minutes, the CTT for viscous tetracaine–treated eyes was significantly (P < 0.001) different from that of saline solution–treated eyes and aqueous proparacaine–treated eyes, but not from that of aqueous tetracaine–treated eyes. The duration of maximum anesthetic effect (defined as the time point at which the CTT was not significantly different from the maximum effect) was 20 minutes for aqueous proparacaine–treated eyes and aqueous tetracaine–treated eyes, and 30 minutes for viscous tetracaine–treated eyes. No corneas retained fluorescein stain at the completion of testing. None of the horses had blepharospasm, conjunctival hyperemia, or chemosis after application of any of the 4 ophthalmic solutions. Viscous tetracaine solution was more difficult to apply because of its high viscosity.

Discussion

Results of the present study indicated that a single application of viscous tetracaine or aqueous tetracaine ophthalmic solution decreased corneal sensitivity significantly from baseline, but to a lesser degree. A recent study by Monclin et al3 revealed a mean CTT of 0.5 cm for equine eyes 5 minutes after application of a single drop of 0.5% tetracaine ophthalmic solution. Ten minutes after application, the mean CTT was 0.75 cm.3 This a is slightly higher CTT measurement than in the study reported here (median, 0.25 cm) at the same time point, but is unlikely to result in a clinically relevant difference. In that study,3 a CTT of 0 cm was achieved at 5 and 10 minutes after application by applying 2 drops of 0.5% tetracaine solution 1 minute apart or by increasing the concentration to 1%. We achieved a median CTT of 0 cm for 30 minutes after the application of the viscous tetracaine solution. This is substantially longer than the 16-minute maximal duration of anesthetic effect achieved after application of 2 drops of 0.5% tetracaine solution.3 The increased duration was likely achieved because of the improved contact time afforded by the more viscous gel formulation,3 but may also have occurred because of the volume of agent applied (1 drop vs 0.2 mL).

Tetracaine has been reported in another study1 to cause chemosis, conjunctival irritation, or pain when administered to dogs. A study8 of human patients found that both proparacaine and tetracaine caused pain upon instillation, but tetracaine caused more pain. Proparacaine and tetracaine also have the potential to create corneal damage if misused4–11 because topical anesthetics are not intended for long-term use. Protracted use can result in neurotrophic keratopathy.12 However, the toxicities associated with these drugs are rarely seen clinically when used at recommended concentrations.

In the present study, horses tolerated the application of all agents equally well, with no chemosis, blepharospasm, or conjunctival hyperemia observed. The study by Monclin et al3 did not reveal any discomfort related to application of tetracaine, although 1 horse developed self-limiting chemosis. The authors did observe punctuate superficial corneal lesions at the completion of the study3 and also hypothesized that those lesions resulted from the esthesiometer filament or the destabilizing effect of tetracaine on the equine tear film.1 We did not, however, observe any corneal lesions at the completion of the study reported here.

Our results after instillation of proparacaine indicate a slightly better anesthetic effect, compared with results in the study by Kalf et al.2 At 10 minutes after application, the median CTT in our study for proparacaine was 0.5 cm. In the study by Kalf et al,2 the shortest reported filament length was 1.25 cm, which was recorded 5 minutes after instillation. The difference in results is likely a reflection of the differences in the individual horses used in each study or may reflect differences in technique, considering that the authors commented that the numerous and lengthy cilia caused difficulty in obtaining the esthesiometry readings.2 The authors also commented that the wind and lighting conditions varied during the study.2 Variations in environmental conditions, breed, and stress have all been reported to cause variation in esthesiometry readings.3
A Cochet-Bonnet esthesiometer was used in the present study to evaluate the effect of the topical anesthetics. The esthesiometer provides an objective evaluation of CTT following mechanical stimulus. The CTT is the pressure at which the majority of touch stimuli causes a blink response. The Cochet-Bonnet esthesiometer has been used to evaluate corneal sensitivity in humans,11 rabbits,12 dogs,13–15 cats,16 and horses17–19 and has been used in multiple studies20–23 to evaluate the efficacy of topical anesthetics. The central corneal CTT measurements obtained before treatment (median, 4.5 cm) are similar to those in horses reported elsewhere by Kalf et al20 (4.82 cm) and Brooks et al19 (4.8 cm) and higher than those reported by Monclin et al1 (2.12 cm) and Kaps et al18 (2.74 cm). In all studies, a Cochet-Bonnet esthesiometer was used and the values reported for the central cornea were compared.

The drawbacks of the Cochet-Bonnet esthesiometer include variability with changes in ambient temperature and humidity; inadvertent changes in the shape of the nylon filament; inadvertent touching of the eyelashes or lid margins, which can stimulate noncorneal sensory nerve receptors to cause the blink reflex; and the provocation of the blink reflex as the instrument approaches the eye. We attempted to minimize these effects by testing all horses under similar conditions. We also examined the nylon filament for defects after each use.

There are some limitations of the present study. As other studies have indicated that the maximal effect of topical anesthetics frequently occurs within the first 10 minutes of application, it would have been ideal to evaluate the effect on corneal sensitivity more frequently during this time period. However, we found horses to be more cooperative if they had a few minutes to calm down after being restrained for application of the anesthetic. If readings were attempted within the first 5 minutes after application of a treatment solution, more head and eye movement occurred, making it difficult to get accurate CTT measurements. In horses that became anxious, it could take several minutes to get an accurate reading. In such cases, the reading obtained may not have been reflective of the actual CTT at the time in question. Also, only horses with normal corneas were included in the present study. Corneal hypesthesia significantly prolongs the duration of action of proparacaine in humans,24 and similar effects may be present in horses with corneal pain. Therefore, the corneal anesthesiometer in clinical patients may be better than that found in horses of the present study.

Topical ophthalmic anesthetic agents are frequently used in veterinary medicine to facilitate performing ocular examinations and minor surgical procedures. Their use is especially valuable in equine patients. The use of topical ophthalmic anesthetics in combination with IV sedation allows procedures such as corneal and conjunctival scraping, removal of superficial foreign bodies, and subconjunctival or intracameral injection to be performed on a standing horse, thereby avoiding the potential risks of general anesthesia. The ability of the viscous tetracaine solution to completely ablate corneal sensation for a period of 30 minutes provides an ample time frame in which to complete required examination or surgical procedures without having to repeatedly apply the topical anesthetic agent. The viscous tetracaine produced more effective anesthesia of the equine cornea, compared with aqueous proparacaine and aqueous tetracaine solutions. All horses tolerated both tetracaine solutions well. The only disadvantage of the viscous tetracaine is that the high viscosity makes application to the equine corneal surface more difficult because it will not spray out through the hub of a needle. The viscous tetracaine comes out as a drop. Therefore, the authors recommend the use of a curved ocular irrigation cannula to facilitate application of viscous tetracaine to the ocular surface.

References


From this month’s *AJVR*

**Prevalence and antimicrobial susceptibility of virulent and avirulent multidrug-resistant *Escherichia coli* isolated from diarrheic neonatal calves**

Robert Barigye et al

**Objective**—To determine the prevalence of selected virulence genes and the antimicrobial susceptibility of multidrug-resistant (MDR) *Escherichia coli* isolated from diarrheic neonatal calves.

**Sample**—97 *E coli* isolates from diarrheic neonatal calves.

**Procedures**—*E coli* isolates were tested via PCR assay for 6 virulence genes and susceptibility to 17 drugs belonging to 9 classes. A 2-sample test of proportions was used to make comparisons between proportions of virulent and avirulent MDR isolates.

**Results**—23 of 97 (23.7%) isolates were virulent, and 74 (76.3%) were avirulent. Of the 23 virulent isolates, 15 (65.2%) were positive for K99, 14 (60.9%) for F41, 12 (52.2%) for STA, 9 (39.1%) for Stx1, 6 (26.1%) for intimin, and 0 (0%) for Stx2. Twenty of 23 (87.0%) virulent isolates expressed ≥ 2 virulence genes, and 3 of 23 (13.0%) were positive for 1 virulence factor. Eight of 23 (34.8%) virulent isolates expressed STA, K99, and F41, whereas 1 of 23 (4.4%) was positive for STA, F41, intimin, and Stx1. The second most frequent gene pattern was Stx1 and intimin. Twenty of 23 (87.0%) virulent isolates were MDR; the highest prevalence of resistance was recorded for the macrolides-lincosides, followed by the tetracyclines and penicillins. Also, 17 of 23 (74.0%) virulent isolates were resistant to sulfadimethoxine, and 10 of 23 (43.5%) were resistant to trimethoprim-sulfamethoxazole. Additionally, 60 of 74 (81.0%) avirulent isolates were MDR.

**Conclusions and Clinical Relevance**—The prevalence of multidrug resistance was comparable for virulent and avirulent *E coli* isolated from diarrheic neonatal calves. Cephalosporins and aminoglycosides had reasonable susceptibility. (*Am J Vet Res* 2012;73:1944–1950)