Degree and duration of corneal anesthesia after topical application of 0.4% oxybuprocaine hydrochloride ophthalmic solution in ophthalmically normal dogs

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Objective—To assess the anesthetic efficacy and local tolerance of topically applied 0.4% oxybuprocaine ophthalmic solution to in dogs and compare its effects with those of 1% tetracaine solution.

Animals—34 ophthalmically normal Beagles.

Procedures—Dogs were assigned to 2 groups, and baseline corneal touch threshold (CTT) was measured bilaterally with a Cochet-Bonnet aesthesiometer. Dogs of group 1 (n = 22) received a single drop of 0.4% oxybuprocaine ophthalmic solution in one eye and saline (0.9% NaCl) solution (control treatment) in the contralateral eye. Dogs of group 2 (n = 12) received a single drop of 0.4% oxybuprocaine ophthalmic solution in one eye and 1% tetracaine ophthalmic solution in the contralateral eye. The CTT of each eye was measured 1 and 5 minutes after topical application and then at 5-minute intervals until 75 minutes after topical application.

Results—CTT changes over time differed significantly between oxybuprocaine-treated and control eyes. After instillation of oxybuprocaine, maximal corneal anesthesia (CTT = 0) was achieved within 1 minute, and CTT was significantly decreased from 1 to 45 minutes, compared with the baseline value. No significant difference in onset, depth, and duration of corneal anesthesia was found between oxybuprocaine-treated and tetracaine-treated eyes. Conjunctival hyperemia and chemosis were detected more frequently in tetracaine-treated eyes than in oxybuprocaine-treated eyes.

Conclusions and Clinical Relevance—Topical application of oxybuprocaine and tetracaine similarly reduced corneal sensitivity in dogs, but oxybuprocaine was less irritating to the conjunctiva than was tetracaine. (Am J Vet Res 2013;74:1321–1326).
properties of oxybuprocaine after application to the human cornea were initially reported in 1955. Although the 0.4% solution was used for many years in the United States, this preparation has been discontinued, and the drug is currently available only in combination with a vial dye (eg, sodium fluorescein or disodium fluorescein) for use in applanation tonometry. Oxybuprocaine hydrochloride 0.4% ophthalmic solution is currently used routinely in Europe for diagnostic and surgical procedures involving the eyes of humans. It also is a topical anesthetic of choice for veterinary ophthalmology. To our knowledge, there have been no data published on the effects of oxybuprocaine ophthalmic solution on corneal sensitivity in dogs.

The Cochet-Bonnet aesthesiometer induces a brief, localized noxious stimulus to the corneal surface. It has been used to quantify corneal sensitivity in humans and domestic animals. The concept for the device is based on the von Frey principle, which states that the force required to distort a long hair when applied axially against the cornea is constant and proportional to the diameter and length of the hair. The Cochet-Bonnet aesthesiometer currently in use has a 0.12-mm-diameter flexible nylon thread, the length of which can be changed from 0.5 to 6 cm to control the intensity of the pressure applied by the tip of the nylon thread to the cornea (ie, the longer the thread, the lower the intensity of the pressure). Mechanical stimuli that evoke corneal sensations correspond to pressures of 11-15 mN/mm², and thin myelinated Aδ fibers in corneal neurons are activated. The Cochet-Bonnet aesthesiometer has been the standard clinical method used to evaluate and monitor the depth and duration of corneal analgesia after topical ocular application of proparacaine, tetracaine, and naltbufine in several species of animals.

The study reported here was designed to evaluate the analgesic effect of 0.4% oxybuprocaine ophthalmic solution on corneal sensitivity in ophthalmically normal dogs and to compare the pharmacological and potential toxic effects of oxybuprocaine with those of 1% tetracaine solution. Our hypothesis was that topical analgesics were unpreserved solutions supplied in 5-mL multidose containers. After each dropper bottle was opened, it was stored at 6° to 8°C and used for only 2 or 3 days to reduce the risk of bacterial contamination of the solution, as has been recommended. After baseline CTT measurements were obtained, dogs of group 2 received a single drop of a 0.4% ophthalmic formulation of oxybuprocaine in one randomly selected eye and a single drop of 1% tetracaine ophthalmic solution in the contralateral eye. Both topical anesthetics were unpreserved solutions supplied in unit-dose applicators.

**Materials and Methods**

**Animals**—Thirty-four healthy adult Beagles (25 females and 9 males) were used in the study. The dogs were university-owned research animals. They were 1 to 11 years old (mean, 4.4 years; median, 3.4 years). All dogs were housed in laboratory animal facilities for at least 3 months before the start of the study. Rooms were maintained at a mean ± SD ambient temperature and relative humidity of 19 ± 1°C and 50 ± 5%, respectively. Dogs were included in the study if they were considered ophthalmically normal on the basis of results of an ophthalmic examination that included slit-lamp biomicroscopy, indirect ophthalmoscopy, and rebound tonometry. Dogs with Schirmer tear test values < 10 mm/min were excluded from the study. All procedures were performed in compliance with institutional and national guidelines in accordance with the European Community Council directive 86/609/EEC.

**Procedures**—Dogs were allocated via a randomization procedure (with a table of random numbers) into 2 groups (22 dogs in group 1 and 12 dogs in group 2). In each group, CTT measurements were obtained by use of a Cochet-Bonnet aesthesiometer for both eyes of each dog immediately before topical administration of solutions (time 0; baseline). Solutions then were topically administered to the dogs, and CTT values were obtained for both eyes of each dog 1 and 5 minutes after topical administration and then at 5-minute intervals until 75 minutes after topical administration.

After baseline CTT measurements were obtained, dogs of group 1 received a single drop of 0.4% oxybuprocaine hydrochloride ophthalmic solution in one randomly selected eye and a single drop of sterile saline (0.9% NaCl) solution (control treatment) in the contralateral eye. Unpreserved oxybuprocaine was supplied in 5-mL multidose containers. After each dropper bottle was opened, it was stored at 6° to 8°C and used for only 2 or 3 days to reduce the risk of bacterial contamination of the solution, as has been recommended. After baseline CTT measurements were obtained, dogs of group 2 received a single drop of a 0.4% ophthalmic solution of oxybuprocaine in one randomly selected eye and a single drop of 1% tetracaine ophthalmic solution in the contralateral eye. Both topical anesthetics were unpreserved solutions supplied in unit-dose applicators.

**CTT measurement**—The CTT measurements were performed with each dog minimally restrained in a sitting position on an examination table. A Cochet-Bonnet aesthesiometer with a 0.12-mm-diameter nylon filament was used to measure sensitivity of the central portion of the cornea, as described elsewhere. Briefly, the tip of the filament was applied perpendicularly to the cornea until a minimal bending of the filament was observed. Initial measurements were obtained with the nylon filament at the maximum length (6 cm); the filament length was then gradually shortened by 0.5-cm increments until a blink reflex was observed. When a blink reflex was noticed, the filament length was then increased by 0.5 cm and measurements repeated until the CTT was determined. The CTT value was the shortest filament length that induced a blink reflex for at least 3 of 5 stimulations. For the posttreatment period, the initial length for the filament was the shortest length that had not induced a blink response during the pretreatment measurement. To minimize variation for the technique, all measurements were obtained by 1 observer (JYD), who used binocular head loupes with 4.0X magnification to accurately determine the initial bending of the nylon filament when the tip was applied to the cornea.

**Evaluation of potential adverse effects**—For dogs of group 2, blepharospasm, bulbar conjunctival hyperemia, and bulbar conjunctival edema were recorded for each eye by 1 observer (AR), who was unaware of the treatment applied to each eye. These variables were subjectively evaluated on a scale of 0 to 3 (0 =
Results

Group 1—Mean ± SD pretreatment CTT values did not differ significantly between the control eyes (1.54 ± 0.41 cm) and oxybuprocaine-treated eyes (1.54 ± 0.40 cm). The mean CTT value remained fairly constant over the observation period in eyes treated with saline solution, and there was no significant change in CTT value after instillation of saline solution, compared with the baseline value (Figure 1). The 2-way analysis revealed that the CTT pattern during the observation period differed significantly (P < 0.001) between the oxybuprocaine-treated eyes and control eyes. Following instillation of oxybuprocaine, mean corneal sensitivity decreased significantly from 1 to 45 minutes, compared with the baseline value. Complete corneal anesthesia (corresponding to a CTT value of 0) was achieved by 1 minute after instillation and was maintained in all 22 oxybuprocaine-treated eyes for a minimum of 15 minutes. The duration of complete corneal anesthesia ranged from 15 to 50 minutes (mean ± SD, 31.6 ± 9.7 minutes) for the 22 oxybuprocaine-treated eyes. The interval for return of corneal sensitivity to baseline values after oxybuprocaine instillation ranged from 25 to 70 minutes (mean ± SD, 52.3 ± 11.4 minutes) among the 22 treated eyes (Table 1).

Group 2—Analysis of mean ± SD pretreatment CTT values revealed no significant difference between the oxybuprocaine- and tetracaine-treated eyes (1.41 ± 0.14 cm and 1.45 ± 0.15 cm, respectively). After treatment, there was a significant decrease in mean CTT value by 1 minute, compared with the baseline value, for both the oxybuprocaine- and tetracaine-treated eyes. Overall, the decreases in CTT value in response to oxybuprocaine and tetracaine ophthalmic solutions were of the same magnitude and duration, and the 2-way analysis revealed that the changes in CTT values over time did not differ significantly (P = 0.066) between the 2 topical anesthetics (Figure 1). Complete corneal anesthesia was achieved by 1 minute after instillation of both treatments, and mean duration of complete anesthesia did not differ significantly (P = 0.52) between oxybuprocaine- and tetracaine-treated eyes (34.5 ± 11.7 minutes and 31.6 ± 10.0 minutes, respectively; Table 1). Similarly, the mean interval until the return to baseline corneal sensitivity was not significantly different (P = 0.90) between the oxybuprocaine- and tetracaine-treated eyes (53 ± 8.7 minutes and 54.5 ± 7.5 minutes, respectively).

Statistical analysis—Data were reported as mean ± SD. In both study groups, changes in CTT between treatments were compared by use of a 2-way analysis that included the main effects of treatment, time, and their interaction. Differences over time were investigated by use of linear contrasts with the Tukey multiple comparison test. For each eye treated with a topical anesthetic, duration of complete corneal anesthesia and interval until corneal sensitivity returned to baseline values were recorded. Duration of complete corneal anesthesia was defined as the time during which there was a lack of a blink response to the maximal corneal stimulation (ie, filament length of 0.5 cm, which was equivalent to a CTT value of 0). Interval until corneal sensitivity returned to baseline values was determined as described elsewhere. A 1-way ANOVA was used to compare duration of complete corneal anesthesia and interval until corneal sensitivity returned to baseline values between the oxybuprocaine- and tetracaine-treated eyes of dogs in group 2. The standard assumption of variance homogeneity was confirmed for each analysis. For all comparisons, values of P < 0.05 were considered significant.

Figure 1—Mean ± SD CTT before (time 0) and after topical application of a single drop of 0.4% oxybuprocaine ophthalmic solution in one eye (black circles) and a single drop of sterile saline (0.9% NaCl) solution in the contralateral eye (white circles) of 22 dogs (A) and a single drop of 0.4% oxybuprocaine ophthalmic solution in one eye (black circles) and a single drop of 1% tetracaine ophthalmic solution in the contralateral eye (white squares) of 12 dogs (B). Values represent the filament length of a Cochet-Bonnet aesthesiometer. Complete anesthesia was defined as lack of a blink response to maximal corneal stimulation (ie, filament length of 0.5 cm), which was equivalent to a CTT value of 0. *Within a treatment group, values differs significantly (P< 0.05) from the value before topical application (time 0; baseline).
ment periods in the present study were not significantly
observe corneal contact.
magnification were used to
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nylon monofilament. Precise observation of corneal
portion of the cornea is achieved indirectly by assessing
measurement of the stimulus intensity to the central

discussion
The purpose of the present study was to determine
the analgesic properties of a topical application of 0.4%
oxibuprocaine to the cornea of ophthalmically normal
eyes of dogs; a Cochet-Bonnet aesthesiometer was used to
quantify changes in corneal sensitivity. The 0.4%
oxibuprocaine eye drops used in the study were pre-
servative-free solutions supplied in a multidose bottle
or unit-dose applicators, which are currently used in
human and veterinary ophthalmology in France. The
Cochet-Bonnet aesthesiometry procedure was similar
to the standard procedure used to evaluate the phar-
macological efficacy of various ocular topical anesthet-
ic in other studies, and the investigators were
careful to control environmental and technical factors
that could affect reliability of the results. To minimize
the effects of ambient temperature and humidity on the
stiffness of the nylon thread of the Cochet-Bonnet aes-
thesiometer, all CTT measurements were performed
on dogs housed in facilities where temperature and hu-
midity were controlled and maintained at consistent
values throughout the study period. Only 1 breed of
mesaticephalic dog was used in the present study to
reduce variation in CTT values possibly linked to dif-
fences in facial conformation among dogs. Because
measurement of the stimulus intensity to the central
portion of the cornea is achieved indirectly by assessing
the extent of bending of the filament, corneal contact
should be detected as the smallest visible bending of the
nylon monofilament. Precise observation of corneal
contact has been conducted in human ophthalmology
by attaching the Cochet-Bonnet aesthesiometer to a
slit-lamp biomicroscope; in the present study, binocu-
lar magnifier loupes with ×4 magnification were used to
observe corneal contact.

The mean baseline CTT values during the pretreat-
ment periods in the present study were not significantly
different within group 1 and group 2 dogs, and they
were extremely similar between dogs of groups 1 and
2. These values were consistent with corneal sensitivity
of canine eyes established with the Cochet-Bonnet aes-
thesiometer and were in accordance with the pretreat-
ment CTT values reported in other investigations on
the effects of topical administration of proparacaine
and tetracaine in healthy dogs.

Analysis of results of the present study revealed that
when 1 drop of preservative-free 0.4% oxyibuprocaine
ophthalmic solution was topically applied to ophthalm-
ically normal dogs, there was rapid and pronounced
corneal anesthesia, as assessed with a Cochet-Bonnet
aesthesiometer. Complete desensitization was evident
at 1 minute after application, and duration of maximal
corneal anesthesia ranged from 15 to 50 minutes (mean
± SD, 31.6 ± 9.7 minutes) for the 22 treated corneas
of group 1 dogs. These findings suggested that diag-
nostic or minor surgical ophthalmic procedures can be
performed with a maximum of comfort for canine pa-
tients for at least 15 minutes after 1 drop of 0.4% oxy-
buprocaine has been applied to an eye. By comparison,
duration of complete corneal anesthesia assessed with
the Cochet-Bonnet aesthesiometer was found to range
from 5 to 20 minutes in human eyes treated with 1 drop
of 0.4% oxyibuprocaine ophthalmic solution. In group
1 dogs, the time until corneal sensitivity returned to
baseline values after oxyibuprocaine administration had
wide intersubject variation, with a range of 25 to 70 min-
utes (mean, 32.3 ± 11.4 minutes). Such duration of action
would be sufficient for most clinical applications in dogs.
In eyes of humans topically treated with 0.4% oxybu-
procaine, wide ranges in recovery of corneal sensitivity
were observed as determined by use of wisps of cotton (10 to
50 minutes) or a Cochet-Bonnet aesthesiometer (30 to
60 minutes). Measurement of corneal sensitivity with a
noncontact corneal aesthesiometer in humans has re-
vealed that the pattern of sensitivity loss (and subsequent
return) after application of 1 drop of 0.4% oxybuprocaine
was a rapid decrease that reached a peak loss at 15 min-
utes, which was followed by a gradual return of corneal
sensitivity over the next 45 minutes.

Currently, the 1% tetracaine ophthalmic prepara-
tion administered to group 2 dogs is available for vet-
erinary ophthalmology in Europe. It was supplied in
preservative-free unit-dose applicators similar to those
used for the oxybuprocaine solution. No significant dif-

<table>
<thead>
<tr>
<th>Variable</th>
<th>Group 1</th>
<th>Group 2</th>
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<tbody>
<tr>
<td>Baseline CTT (cm)</td>
<td>1.54 ± 0.41</td>
<td>1.41 ± 0.14</td>
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<tr>
<td>Duration of complete corneal anesthesia (min)</td>
<td>31.8 ± 9.7</td>
<td>34.5 ± 11.7</td>
</tr>
<tr>
<td>Interval until return to baseline sensitivity (min)</td>
<td>52.3 ± 11.4</td>
<td>55.0 ± 8.7</td>
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Dogs of group 1 (n = 22) received a single drop of 0.4% oxybuprocaine ophthalmic solution in one eye and saline (0.9% NaCl) solution (control treatment) in the contralateral eye. Dogs of group 2 (n = 12) received a single drop of 0.4% oxybuprocaine ophthalmic solution in one eye and 1% tetracaine ophthalmic solution in the contralateral eye. The CTT of each eye was measured before topical application (time 0, baseline), 1 and 5 minutes after topical application, and then at 5-minute intervals until 75 minutes after topical application. Complete corneal anesthesia was defined as lack of a blink response to maximal corneal stimulation (ie, filament length of 0.5 cm), which was equivalent to a CTT value of 0. — = Not applicable.
ferences in onset, depth, and duration of corneal desensitization were found between the tetracaine- and oxybuprocaine-treated eyes in group 2 dogs. Although results from group 1 and group 2 dogs were not compared, similar corneal anesthetic properties were evident between the 2 oxybuprocaine preparations used in the study groups (Table 1). Results of the present study indicated that in ophthalmically normal dogs, the depth and duration of corneal anesthesia after administration of 1 drop of 0.4% oxybuprocaine solution or 1% tetracaine solution are extremely similar to those reported after administration of 1 drop of 0.5% proparacaine solution. Similarly, duration of corneal anesthesia was almost the same after topical administration of tetracaine (9.4 minutes) or proparacaine (10.7 minutes) in humans. Anesthetic properties of the 1% tetracaine ophthalmic solution applied in the present investigation were consistent with those reported in a study conducted to evaluate the pharmacological effects of 1% tetracaine combined with 0.1% phenylephrine on corneal sensitivity in dogs by use of a Cochet-Bonnet aesthesiometer. However, in that study, complete anesthesia of the cornea was detected 10 to 15 minutes after administration, whereas it was detected by 1 minute after treatment in the study reported here. Similar delays of 5 and 10 minutes until maximum corneal anesthetic effect after administration of 1 drop of 1% and 0.5% tetracaine solutions have been reported in horses. These differences might be related to the rapidity with which a drug diffuses through the corneal epithelium, which depends on the ratio of the ionized (hydrsoluble) and nonionized (liposoluble) forms of the molecule and is directly influenced by the pH of the ophthalmic preparation. The oxybuprocaine and tetracaine ophthalmic solutions of the present study had pH values of 4.26 to 4.55 and 4.64 to 4.71, respectively, which were in agreement with data in other reports. Thus it seems unlikely that differences in the drug volume instilled were a source of intersubject variation in the anesthetic effects observed. Both the type of container and design of the tip can affect repeatability of drop size, which is one of the determinants of the extent of ocular effects. Although we did not evaluate variation in the volume of the drops expelled by the containers in the present study, we can hypothesize that variation in the volume instilled had a possible effect on the degree and duration of corneal anesthesia among the dogs. Intra-individual differences probably also accounted for the intersubject variation in the results because of similar observations after topical application of oxybuprocaine in eyes of humans and tetracaine in eyes of dogs.

To our knowledge, the study reported here is the first in which objective information has been obtained on the analgesic effects for topical administration of 0.4% oxybuprocaine hydrochloride ophthalmic solution to healthy eyes of dogs. Results indicated that onset, depth, and duration of corneal anesthesia provided by this anesthetic agent were not different from those observed after administration of 1% tetracaine solution to ophthalmically normal eyes of dogs. These results suggested that oxybuprocaine is appropriately suited as a topical ocular anesthetic agent for dogs that can be used in a broad spectrum of clinical settings and with fewer risks of conjunctival changes, compared with those for tetracaine.

References


