COMPARISON OF EFFICACY AND DURATION OF TOPICAL ANESTHETICS ON CORNEAL SENSITIVITY IN CLINICALLY NORMAL HORSES

by

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Abstract

Objective- The purpose was to compare the efficacy and duration of 0.5% proparacaine, 0.5% bupivacaine, 2% lidocaine, and 2% mepivacaine on corneal sensitivity in clinically normal horses.

Animals- 68 clinically normal horses

Procedures- In group 1, 60 horses from the Kansas State University horse unit were assigned to receive one topical anesthetic in a completely randomized design. In group 2, 8 privately owned horses were sequentially treated with each of the topical anesthetics in random order with a one week washout period between drugs. Corneal sensitivity was assessed by corneal touch threshold (CTT) measurements which were taken with a Cochet-Bonnet aesthesiometer before anesthetic application (T0), 1 minute after (T1), every 5 minutes until 60 minutes (T5-T60), and then every 10 minutes until 90 minutes (T70-T90) after application. General linear mixed models were fitted to CTT in each design in order to assess the effects of topical anesthetics over time, accounting for repeated observations within individual horses.

Results- Corneal sensitivity, as determined by CTT measurements, decreased immediately following application of the topical anesthetic, with persisting effects until T35 for proparacaine and mepivacaine, T45 for lidocaine, and T60 for bupivacaine. Maximal CTT reduction was achieved following application of bupivacaine or proparacaine, while mepivacaine was least effective.

Conclusions and Clinical Relevance- All topical anesthetics reduced corneal sensitivity, though maximal anesthesia and effect of duration differed between drugs. For brief corneal anesthesia, 0.5% proparacaine or 2% lidocaine appeared adequate, while 0.5% bupivacaine may be most appropriate for procedures requiring longer periods of corneal anesthesia.

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Chapter 1 - Literature Review

The Cornea

Corneal Anatomy

Positioned on the anterior surface of the eye, the cornea of most humans and domestic animals is a unique, minimally cellular, and avascular structure which comprises an essential component of the visual system. It functions to provide structural support to the eye as well as refraction and transmission of light.[1,2] The uniqueness of this site is its biological requirement for maintenance of an optically clear and normal window.[3] Three basic tissue layers comprise the cornea: the epithelium, stroma, and endothelium. The interactions of each one of these layers with the surrounding environment is crucial to cell survival and corneal architecture.[4]

Epithelium

The epithelium is found on the most anterior surface of the cornea and consists of layers of nonkeratinized stratified squamous epithelial cells. The cells connect together by means of short, interdigitating processes that are adherent at desmosomes.[3,5] The outermost surface of the epithelial cell layer forms numerous small membrane folds called microvilli and microplicae. These projections measure only a few tenths of a micron in height, but may serve to increase oxygen absorbance and retain the tear film on the surface of the cornea.[1] In addition to retention of the tear film, these cells provide a maintenance function to the barrier between the inner layers of the cornea and the tears. In most species, the epithelium undergoes a turnover every seven days by sloughing the outer layer into the tear film.[6] Immediately below the surface epithelium is a layer of polygonal-shaped cells termed the wing cell layer.[1] Finally, beneath the wing cells lies the germative cells known as the basal cell layer, which are the only cells in the cornea to

undergo mitosis.[1] In humans, giraffes, whales, birds, and primates the basal cell layer is a cuboidal shaped cell layer that rests atop a fibrillar lamina known as Bowman's membrane. Although not a true membrane, this structure is produced by extracellular secretions of the basal cells and represents the outermost layer of the corneal stroma.[1,3,6] In those species without a Bowman's membrane, the basal cells sit upon a thin basal membrane consisting of tightly cross-linked fibers of laminin and heparan sulfate.[3]

Stroma

The corneal stroma is situated beneath the basement membrane of the epithelium and provides approximately 90% of the corneal thickness.[1] This layer includes keratocytes which occupy around 3% of the volume, fibroblasts, and some neural tissue. Keratocytes function to maintain the normal stromal composition and extracellular matrix, but when injured they become fibroblastic and produce stromal collagen.[1] Transparent collagen fibrils, spanning the entire corneal diameter, are arranged into bundles in lamellar planes in a precise organization necessary to provide corneal clarity.[2] In each layer, the direction of the fibers changes so as to cross at diverse angles with those of successive layers.[1,2,5,7] The lamella are closely attached to each other with interlamellar spaces smaller than the wavelength of light that eliminate any significant reflections of light at the surface of individual lamella.[5] This special alignment allows for 99% of light entering the cornea to pass through without scatter.[8]

Endothelium

The endothelium is a single layer of large, flattened squamous cells that lines the inner surface of the cornea and secretes a basement membrane termed Descemet's membrane. Descemet's membrane is 5-10 μm thick, and though it serves to separate the stroma from the endothelium, there are collagen fibrils from the stroma embedded in Descemet's.[7]

Immunohistochemical studies have determined that the composition of Descemet's membrane includes fibronectin, type IV collagen, and laminin.[5] Early in life, this layer appears homogenous, but with age becomes thickened by continuous secretions from the endothelium and more fibrous in appearance.[3] This basal lamina increases thickness at a rate of about 1 micron per 10 years of age in humans.[1]

Endothelial cells perform an important corneal physiologic function. These specialized cells contain pump mechanisms and intracellular spaces located amid the cells which permit controlled fluid passage between the stroma and the anterior chamber.[1] Unlike the epithelium, contact between adjacent cells in the endothelium is not occlusive and the cell junctions are known to be leaky. However, the cells of this layer are responsible for the corneal stroma's relative state of deturgesence needed to help maintain corneal clarity and are the most metabolically active cells of the cornea. The endothelial cells are not regenerative and over time a gradual loss of these cells occurs without an ability to replace them. To make up for the loss, neighboring endothelial cells enlarge in size to maintain apposition with adjacent cells in an effort to prevent direct access of aqueous to stroma.[1] This continues to occur with further endothelial cell loss until a critical level is reached at which the remaining cell density cannot compensate for the losses, resulting in stromal fluid uptake and visible corneal edema.

Corneal Innervation

Vision plays an important role in most animals' ability to obtain information from the environment, and because of this, the structures maintaining the visual pathway are provided with specialized protective mechanisms. Evidence for this is that the cornea is one of the most highly innervated structures in the body with a nerve density 300-600 times that of skin.[9] Compared to other regions of the ocular adnexa, the cornea possesses the richest sensory

innervation enabling it to sense the slightest encounter with potentially injurious physical or chemical stimuli.[10] Corneal innervation has been extensively studied in humans and other species through a multitude of techniques including; various histological stains, electron and light microscopy, immunohistochemistry, and in vivo confocal microscopy.[11-17]

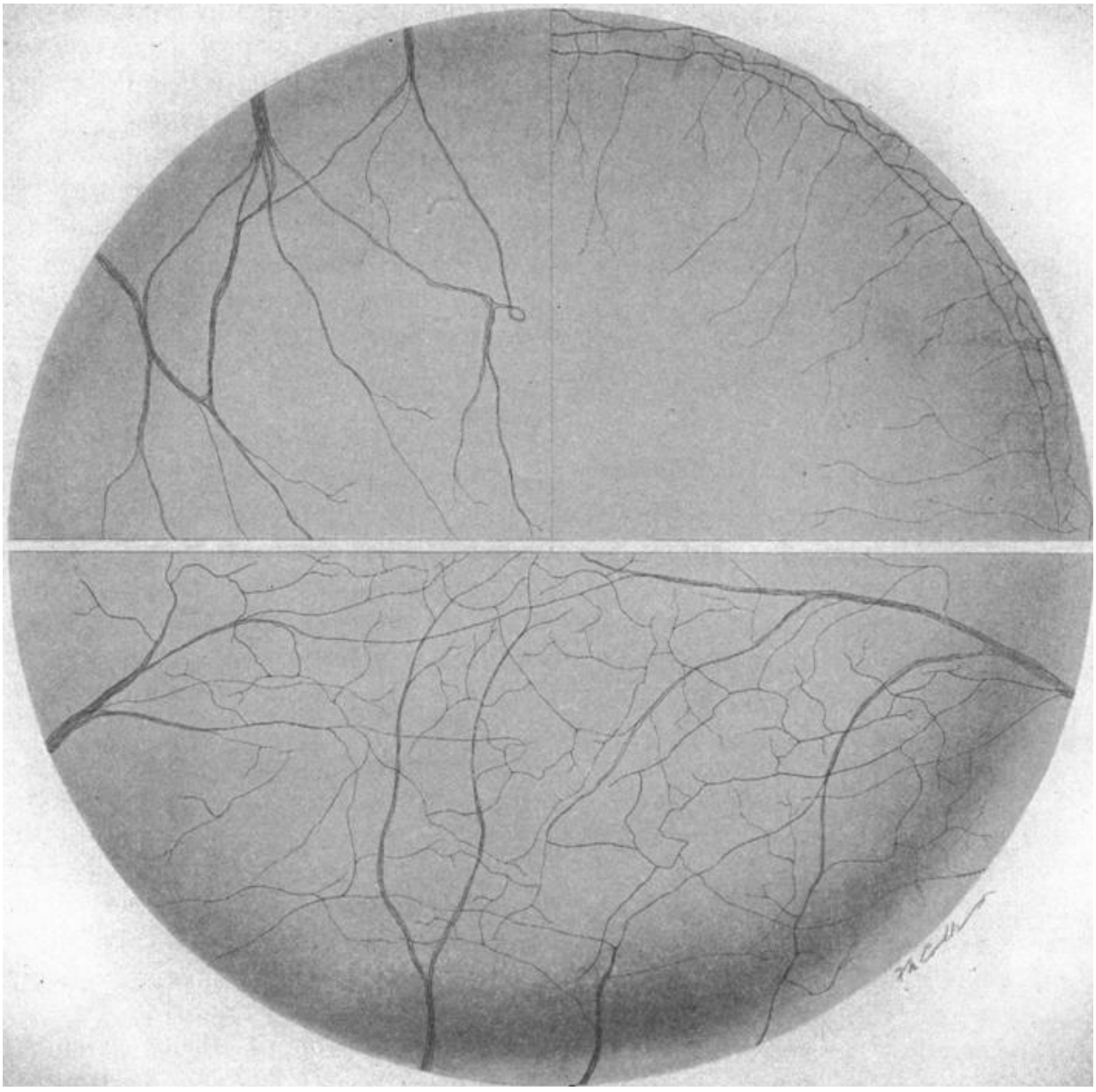
Corneal nerves serve to maintain the integrity of the ocular surface by promoting corneal epithelial homeostasis through release of trophic substances and activating brainstem circuits which stimulate reflex tearing and blinking.[18,19] Though these nerves are comprised of both sensory and autonomic fibers, sensory fibers dominate and are derived from the antero-median area of the trigeminal ganglion.[20] From the ophthalmic branch of the trigeminal nerve, fibers exit to form the long posterior ciliary nerves which enter the globe medial and lateral to the optic nerve head, coursing forward through the suprachoroidal space. Before reaching the limbus these nerves branch into smaller bundles repetitively and anastomose extensively with branches of the short ciliary nerves to form uniformly distributed nerve bundles that approach the limbus radially from all directions.[18,21] Varying numbers of nerve trunks have been shown to enter the limbus with 10-18 in dogs and rabbits,[9,12,21] followed by 18-21 in cats,[22] and humans have the most nerve trunks present at the limbus with 30-80 (**Figure 1.1**).[11,15,18,23] To date no studies have been performed to document the corneal innervation of the equine eye.

After arriving at the limbus the unmyelinated axons have a structure very similar to a peripheral nerve as they are of large diameter, have irregularly spaced collagen fibrils, and are surrounded by a fibrous endoneurial layer.[1] Some axons are myelinated for a distance into the cornea of 1-2 mm, but then must lose this covering in order to not alter refraction of light and maintain corneal clarity.[14,16] Despite a lack of myelin covering, the nerves are often accompanied by Schwann cell sheaths up to the level of the basal lamina.[14]

From the limbus, the nerve trunks penetrate the midstroma of the cornea and begin a series of repetitive branching into smaller stromal nerves. These smaller nerves anastomose with those from other nerve trunks to form a moderately dense mid-stromal plexus in the periphery of the cornea.[15,18] Nerves then continue superficially to reach the anterior stroma of the cornea and anastomose again with neighboring nerves to form a subepithelial plexus. The nerves at this level are noted to course seemingly randomly in all directions to form an extensive grid-like meshwork of interconnected nerves (**Figure 1.1**).[11,14,16,18]

A smaller number of these nerves will leave the subepithelial plexus and again travel anterior to penetrate the basal lamina and branch to run parallel to the basal epithelium, forming the subbasal plexus.[16] It has been calculated that approximately 5400-7200 nerve bundles are present in the subbasal plexus of humans with a diameter of the nerves varying between 0.05 - 2.5 μm.[14] Smaller branches from the subbasal plexus then course into the epithelium and are oriented perpendicular to the corneal surface.[21,24] If the assumption is made that each of the nerve fibers present in the human cornea gives rise to 10-20 nerve terminals in the epithelial layer, the number of free nerve endings in the cornea could range from 315,000 – 630,000.[14] The presence of so many nerves positioned across the cornea ensures that any noxious stimuli can be detected and immediate action taken to prevent damage to this important structure.

Figure 1.1 Schematic of corneal innervation in the rabbit. Upper left illustrates large corneal nerve trunk fibers as they enter at the limbus. Upper left shows minor innervation provided by conjunctiva and sclera. Lower half of image displays the highly branching pattern of the nerve trunks as they branch and migrate toward the central portion of the cornea. Image from Zander E, Weddell G. Observations on the innervation of the cornea. *J Anat* 1951;85:68-99.



Corneal Aesthesiometry

The cornea is the most sensitive tissue in the body and is able to perceive touch, warmth, cold, and pain.[23,25] Corneal aesthesiometry is a means to assess corneal touch threshold (CTT), or the amount of pressure necessary to stimulate the blink reflex, and is inversely proportional to corneal sensitivity. Estimation of corneal sensitivity can be necessary to diagnose and monitor certain eye pathologies, assess efficacy of topically applied drugs, or to evaluate progression of corneal healing after surgery.[23] A small piece of cotton wool strand has often been used clinically to stimulate touch responses, but this simple device does not allow for quantification. Different techniques and instruments have been developed over the years to quantify sensitivity of the cornea with the earliest measurement recorded by Dr. Frey in 1894.[23,26] Stimulation of the corneal touch threshold was performed using horse hairs of precise lengths and diameters that were attached to a glass rod using wax.[23] As pressure was applied by the hair pressing against the corneal surface, a counter pressure was exerted by the elastic nature of the hair in an attempt to elicit a blinking response.[26] As the length of the hair increased, the amount of pressure exerted upon the cornea decreased. Testing was initiated with the longest hair length and shortened until a blink response was noted. The hair noted to cause the stimulation was then applied to a scale to obtain an estimate of the amount of pressure applied and thus find the corneal touch threshold. Dr. Frey's technique proved to be impractical because of the number of varying test-hairs necessary as well as the lack of power consistency between hairs.[26] Other devices were developed including Nafe and Wagoner's aesthesiometer which used metal rods instead of hairs, but a clinically useful device was not available until 1955 when Boberg-Ans created an aesthesiometer that uses nylon thread of constant diameter.[23,26] The length of the nylon filament can be altered by sliding the filament out of its specialized holder to adjust amount of pressure

applied.[26] Benefits of this device included ease of use and good reproducibility of values between users.

In 1960, Cochet and Bonnet produced a corneal aesthesiometer which was a modification of the Boberg-Ans, and has become the most clinically used of all aesthesiometers (**Figure 1.2**).[12,22,23,27-29] This device is comprised of a metal cylinder encasing a nylon monofilament with a fixed diameter of 0.12 mm. Despite the small diameter of the filament, the tip is able to stimulate around 100 nerve endings over 4-10 corneal epithelial cells.[9,12,22,29] The filament can be adjusted from 6.0 to 0.5 cm in exposed filament length which corresponds to a pressure when applied to the cornea of 11 to 200 mg per 0.0113 mm^2. Adjustments to filament length are made in 0.5 cm increments as the filament is shortened until a consistent blink response is noted **(Figures 1.3-1.5)**. As with other aesthesiometers, decreasing the length of the filament results in more pressure applied to the corneal surface. The length of filament required to stimulate the blink response is recorded as the corneal touch threshold and can be converted to a pressure reading using the table given with the aesthesiometer (**Table 1.1**). Other corneal aesthesiometers have been developed which are more sophisticated,[30-33] but in corneal aesthesiometry accuracy is not directly proportional to the sophistication of the instrument.[23]

Figure 1.2 Cochet-Bonnet Corneal Aesthesiometer

Figure 1.3 Application of nylon monofilament to central corneal surface with longest filament length (least pressure). Care is taken not to stimulate eyelashes or sensory hairs.

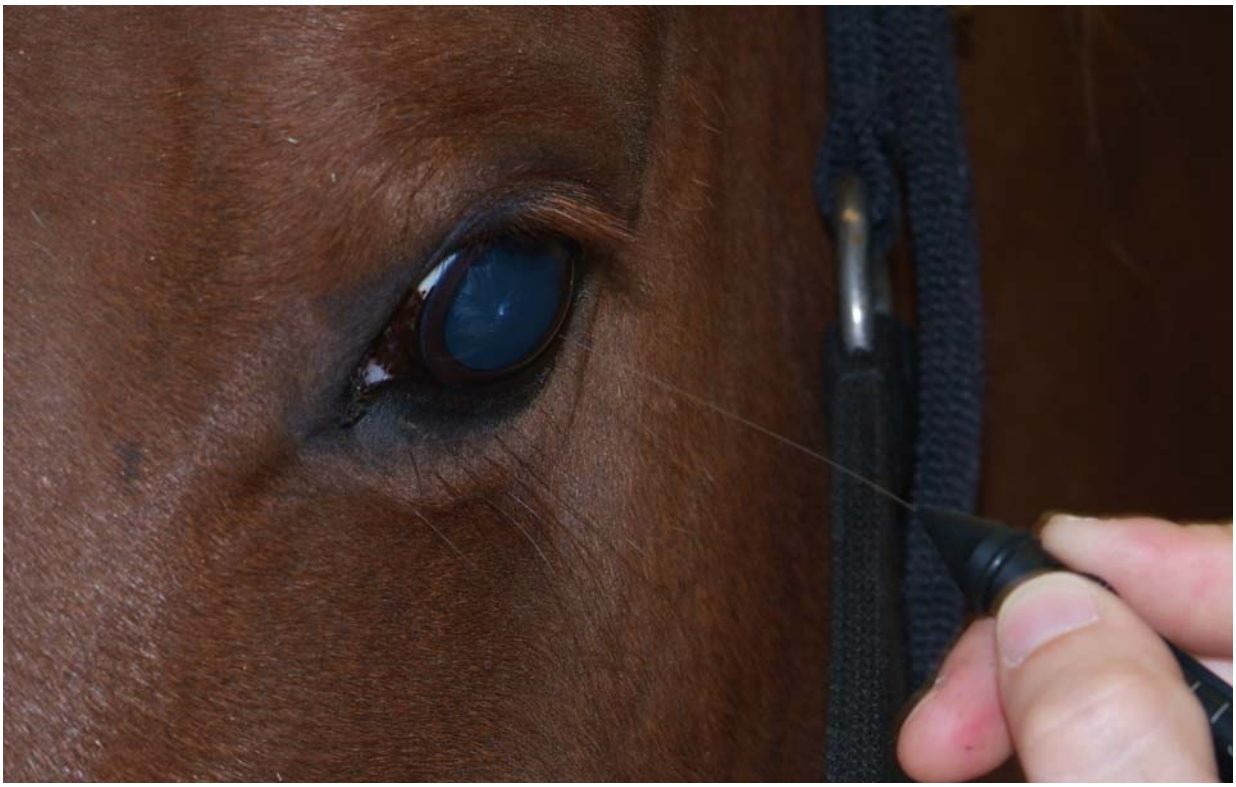


Figure 1.4 Application of nylon monofilament to central corneal surface with shorter filament length (more pressure).

Figure 1.5 Stimulation of blink reflex as corneal touch threshold is achieved.

Table 1.1 Conversion table of measurements made with the aesthesiometer. Nylon monofilament size S= 0.0113 sq. mm (0.12 mm diameter)

Filament length mm	**60**	**55**	**50**	**45**	**40**	**35**	**30**	**25**	**20**	**15**	**10**	**5**
Mean values of pressures mm/g/S	**11**	**12**	**13**	**16**	**21**	**27**	**36**	**52**	**75**	**100**	**145**	**200**
Mean values of pressures in g/ mm^2	**0.96**	**1.08**	**1.16**	**1.40**	**1.84**	**2.40**	**3.20**	**4.60**	**6.64**	**8.54**	**12.84**	**17.68**

Physiological and environmental effects on CTT

With the advent of calibrated corneal aesthesiometers, the ability to detect and study the effects of ocular and systemic diseases on corneal sensitivity has been furthered. Despite attempts at standard use protocols, variability is present in both the patients and the environments in which testing occurs making assessment of corneal aesthesiometry a subjective test. Despite this, the subjective patient response represents the criterion against which the objective measurements are compared, and is considered the most valid psychophysical method for determining corneal sensitivity in humans.[29]

Apprehension

Measurement of corneal touch threshold can create apprehension on the part of the subject being measured, especially when measuring the central cornea. This is well recognized in human test subjects but has not been documented in animals.[23,29] In 1956, Bonnet and Millodot measured corneal sensitivity in the center of the cornea as well as peripherally under normal lighting and compared the values to those taken using only infrared lighting so as to be in total

darkness. The results showed that a significant increase (mean 18%) in corneal touch threshold for the center of the cornea was recorded when the patient was in normal lighting compared to darkness where the patient could not see the device.[23] In addition, apprehension of human test subjects can lead to difficulty in determining whether they felt the stimulus or not.

Diabetes mellitus

Studies have shown a decreased corneal sensitivity as measured by corneal aesthesiometry in humans, dogs, and rats with diabetes mellitus.[23,34-38] Correlations have been made as to the length of time diagnosed with diabetes and the level of desensitization,[35,39] as well as the overall control of diabetes.[37] This desensitization is believed to be a small part of a diffuse neuropathy which affects the peripheral sensorimotor nervous system of diabetics as a reduction in subbasal corneal nerve density has been observed.[35] With diabetic neuropathy, segmental demyelination and axonal degeneration are seen but the exact cause is unknown.[40] Three main theories in humans have surfaced as possible causes for the neuropathy and include ischemic events or infarcts, slowed axonal transport, and altered glucose metabolism affecting corneal epithelial and neuronal function.[34] Of these, no consistent cause can be identified in a majority of patients.

Contact lens wear

The number of people wearing contact lenses daily is estimated to be around 150 million,[41] making the effect of wear on corneal sensitivity of great importance. Corneal aesthesiometry was initially used in an effort to predict the tolerance and success of wearing contact lenses, but other factors proved to more appropriately determine success rate including individual motivation.[42,43] Decreases in corneal sensitivity with contact lens wear was initially thought to be the result of corneal edema and metabolic disturbances as the corneal thickness is

increased in patients using both hard and soft contact lenses.[44] Microscopic examination has shown that a decrease in nerve fiber bundle density is not likely to be the cause.[45] Further examination led to the theory that despite minor increases in corneal thickness, the decreased sensitivity most likely arises from sensory adaption as a consequence of continuous mechanical stimulation.[46] The decreased corneal sensitivity appears to be beneficial as it allows for increased comfort while wearing the lenses.[46,47] Also noted is that the sensitivity recovers rapidly once the lenses are removed, with recovery after short term use taking several hours. Complete recovery may take months, and is directly related to the number of years the contact lenses have been worn.[23] This phenomenon of diminutive sensitivity was first noted in 1955 with use of hard contact lenses,[26] but since then has been demonstrated with soft contact lens use as well.[44-46,48] Factors seemingly affecting corneal sensitivity include the type of lenses, number of hours worn per day, number of years of wear, and whether daily or extended-wear lenses were used.[23]

Effect of age and hormonal status

Corneal sensitivity can also be affected by individual characteristics and among different age groups. Previous testing of corneal sensitivity in healthy neonatal foals and crias have shown that they have a greater sensitivity as compared to adult horses and alpacas respectively.[49,50] In humans, though no significant decrease in sensitivity is typically noted until 50 years, after surpassing this age corneal sensitivity dramatically declines over the next 15 years to half of its previous level.[23,26] In animals, a decrease in sensitivity with age has also been documented and is attributed to a decrease in the levels of the neurotransmitter responsible for corneal nerve transduction, acetylcholine.[23]

Previous reports have shown that the sex of an individual does not lead to a difference in corneal sensitivity, but hormonal status may affect females.[33,51,52] During premenstrum, females

begin to retain fluid which leads to a generalized, but subtle edema and is evidenced to cause a decreased tactile sensitivity of the fingers.[53] In addition, an increase in intraocular pressure is noted in females with glaucoma during this time which alters corneal sensitivity.[26,54] Increased corneal thickness due to generalized fluid retention has been observed in pregnant females,[55] and so it is assumed that both increased corneal thickness and increased intraocular pressures may be to blame for the variation in sensitivity noted in these individuals.[23,51] Despite not knowing the exact mechanism, the effect of hormones on corneal sensitivity is further revealed when comparing corneal touch thresholds in females who are on oral contraceptives to a matched population of females with altered hormone status as well as male test subjects. Results show a significant decrease in corneal sensitivity in females during menstruation compared to females on oral contraceptives and males.[51]

Ambient temperature and humidity

Most diagnostic testing for corneal sensitivity takes place in controlled settings with relatively stable environmental conditions. Despite this, variations in humidity and ambient temperature can cause alterations in sensitivity by affecting the cornea or aesthesiometer.[56-58] A decline in ambient temperature from 22 to -14°C (72 to 7°F) leads to a 9 fold reduction in corneal sensitivity presumably due to a decrease in corneal metabolism and function.[23] This sort of variation in temperature is unlikely in a clinical setting, but stresses the importance of the environment on sensitivity recordings.

When assessing the effect of humidity on corneal sensitivity, several possibilities exist. One of such is that humidity, as well as ambient temperature, can affect the rigidity of the filament used in the corneal aesthesiometers.[23] By influencing the rigidity of the filament, the normally consistent pressure exerted by a specific length of filament is altered leading to

inaccurate sensitivity recordings.[57] Another way humidity can affect corneal sensitivity is through changes to the precorneal tear film.[33] The tear film keeps the epithelial cells moistened, preventing damage to the surface cells as well as providing nutrition. In environments with low humidity, the precorneal tear film is predisposed to dehydration faster, which leaves the corneal surface altered. If exposed chronically because of an abnormal tear film, the cornea becomes irritated and corneal sensitivity can be distorted.[58]

Local Anesthetics

History of Local Anesthetics

Local anesthetics have been used in the clinical setting for over 100 years to provide pain relief but it was not until the last few decades that their mechanism of action was discovered. The first drug used as a local anesthetic was cocaine and its use was discovered by Austrian ophthalmologist Carl Koller in 1884. Dr. Koller was experimenting with the drug and noticed a numbing effect on the tongue after swallowing. This, paired with the findings of pain relief when used by his friend Sigmund Freud, led him to believe it could effectively desensitize the cornea.[59] To test the drug they dissolved it in water and applied it topically to frog corneas. Following successful tests, Koller and Freud proceeded with application to their own eyes and used pins to touch their corneas while staring in a mirror and noticed a lack of touch and pain.[60] In addition to the corneal anesthesia provided, cocaine provided widening of the palpebral fissure, pupillary dilation, and a slight paresis of accommodation.[61] Koller expanded cocaine's use for ocular anesthesia by using it in subconjunctival injections for procedures such as cataract surgery and iridectomies.[62] After Koller and Freud reported their findings, a neurologist named Leonard Corning began experimenting with 2% cocaine for use as an epidural anesthetic.[63] Despite the disadvantages of high toxicity, inability to sterilize properly, short duration, cost, and

addictive nature cocaine was used commonly for many years. It was not until 1904 that the anesthetic properties of cocaine were found to be attributed to its benzoyl acid group. From this, Alfred Einhorn synthesized procaine which failed to take off in use because of its lack of penetration and short duration. A majority of topical anesthetics used today end in "caine" because they contain a benzene ring linked via an amide or ester to an amine group.[60]

Action Potential Propagation

The neuronal axon membrane is comprised of a double layer lipoprotein surrounded by inorganic ions. This membrane provides a selective permeability to ions setting up the electrical potential across the membrane of -70 mV, which is near the potassium equilibrium potential.[64,65] To maintain this potential in the resting state, sodium ions are kept on the outside of the membrane by use of voltage-gated ion channels located along the length of the neuron. These channels are normally closed when the membrane potential is at resting state and only open after a stimulus is determined. Once a sensory nerve obtains a stimulus, the ion channels open and allow an inward flux of sodium ions leading to changes in the electrochemical gradient and depolarization.[64] Depolarization of the neuronal membrane leads to further activation of ion gated channels and allows the influx of sodium ions quickly, becoming greater than the potassium ion outrush until reaching the threshold for voltage-gated sodium channel activation.[60,64] Once this threshold occurs an action potential is produced in an all-or-none process. This occurs along the entire pathway, reversing the potential from negative to positive until reaching the ultimate target destination where the impulse is translated. Once the impulse has been propagated, the ion channels rapidly inactivate. As the sodium channels close, sodium ions are transported out of the membrane and potassium channels are activated. This leads to an inward flux of potassium ions which tends to surpass the resting potential and allow for slight

hyperpolarization so that the action potential cannot travel backwards. After this refractory period, the concentration of ions returns to the resting state until another impulse is sent.

Mechanism of Action

The true mechanism of action is not fully understood for local anesthetics.[66] Most local anesthetics used today are presumed to work by a similar root mechanism of action which entails a reversible blockade of signal transduction at the level of the sodium channel protein with no permanent damage.[64] At clinically relevant concentrations of anesthetics, potassium and calcium channels will also be affected but to a lesser degree.[60] When compared to general anesthetics, the level of interaction with these channel proteins is greater with local anesthetics and the side effects are fewer, which makes their use attractive. The nerve blockade can occur at varying levels along the neural pathway, including the synaptic terminals, but the neuronal membrane is the most common.[64]

After administration, local anesthetics bind to a hydrophilic site within the sodium channel on the inner surface of the cell membrane, inhibiting nerve impulse conduction by stabilizing the axonal membrane.[65] The drug must first pass through the cell membrane as an uncharged base in order to reach the intracellular site. Once inside, the uncharged base is protonated and the charged cation binds to the receptor site.[65] Repetitive stimulation of nerve fibers increases the binding affinity of the receptor site and facilitates development of a reversible neural blockade.[60,64,67] Without conduction along the axon, the brain is kept from detecting the painful stimuli.

Properties Affecting Efficacy and Duration

Most local anesthetics used today are tertiary amines linked either by ester or amide bonds to aromatic residues.[68] These drugs are typically weak bases and are insoluble in water so

they are prepared as salts, making them stable at an acidic pH. The tertiary amine readily accepts protons and is hydrophilic while the aromatic ring is lipophilic. Substitution of alkyl groups on the ring or tertiary amines leads to an increase in lipid solubility.[65] Three main characteristics help to identify their clinical efficacy and duration. These include their lipid solubility, amount of protein binding, and their dissociation constant. Paired with this, the components which comprise most local anesthetic molecules help to contribute to these distinct properties including the lipophilic aromatic ring and a terminal amine.[65,67]

The aromatic ring serves to improve the lipid solubility of the drug, leading to greater diffusion through neuronal membranes. This correlates with an increased amount of administered drug reaching the site of action, or potency.[67] The terminal amine group allows the drug to exist in either a water-soluble or lipid-soluble state.[65] Most local anesthetics are formulated to be in stable, water-soluble state at the time of injection by having them exist at an acidic pH, despite this form not being able to penetrate the neuronal membrane. Once exposed to the more neutral physiologic pH of the site of action, the drug is altered to enhance penetration.

The pH at which the concentration of the lipid soluble molecules is equal to that of the water soluble molecules is defined as the dissociation constant (pK_a). The pK_a of all local anesthetics is > 7.4 (physiologic pH), and so a greater proportion of the molecules exist in the water soluble form when injected into tissue having a normal pH.[67] Once exposed to the normal physiologic pH in the target tissue, the proportion of lipid soluble molecules is increased as the amount of non-ionized particles amplifies, allowing for improved penetration of the membrane.[65,67] How quickly this occurs is correlated with time of onset of action for each drug.

Table 1.2 Selected pH and pK_a values for local anesthetics

Local anesthetic	pH	pK_a

0.5% Proparacaine	**4.6**	**9.1**
2% Lidocaine	**6.5**	**7.7**
0.5% Bupivacaine	**5.6**	**8.1**
2% Mepivacaine	**5.8**	**7.6**

Duration of action is correlated with the amount of protein binding for each drug. As the amount of protein binding increases, the affinity for protein within the sodium channel increases leading to a greater duration of blockade.[67] Bupivacaine has a high degree of protein binding at 96% which is believed to be the reason for the increased duration of action when compared to lidocaine with a protein binding of around 70%.[65,67,69]

Classes of Local Anesthetics

Amides

The class of local anesthetics typically used for injectable local anesthesia is amides, although certain ones can be used topically.[65,70-72] Commonly used drugs belonging to this class include lidocaine, bupivacaine, mepivacaine, procaine, etidocaine, and ropivacaine.[65,67,68,70] After injection, amides cause peripheral vasodilation which accelerates systemic absorption and lessens the effect. Despite this, the lipophilic amides tend to have a longer duration of action than the esters due to their greater degree of protein binding.[65] The majority of amide molecules are then cleared from the body through enzymatic hydrolysis.[68]

Lidocaine is used for local infiltration and intravenous regional anesthesia as well as central nerve blocks. Because of a pK_a of 7.7 and a protein binding of 65%, lidocaine has a rapid onset and intermediate duration of action of one to two hours.[65,69,73] This is in comparison to

bupivacaine which has a slower onset but a duration of action 2-3 times that of lidocaine. The differences are in the higher degree of protein binding (96%) and pK_a (8.1).[65,71,74] Between these two drugs lies mepivacaine which has a fairly rapid onset and slightly longer duration of action than lidocaine (90-180 min). Benefits of mepivacaine over lidocaine and bupivacaine include decreased tissue irritation and higher therapeutic indices.[65,70,75]

Though more commonly used as injectable local anesthetics, some amides are applied topically to the ocular surface. Lidocaine is used in both a topical aqueous solution and more recently as a Food and Drug Administration (FDA) approved topical ophthalmic gel (3.5% lidocaine gel, Akten®) for providing non-invasive local anesthesia during ophthalmic procedures and surgeries.[72,76] Other amides used topically for corneal anesthesia include bupivacaine, ropivacaine, and mepivacaine.[70,71,77,78] Although the effects do not last as long topically as when injected locally, amides can be used to provide adequate corneal anesthesia for most short duration procedures.[72]

Esters

Esters are a group of local anesthetics used for topical corneal anesthesia most commonly. These include proparacaine, tetracaine, oxybuprocaine, and cocaine.[72,79] Of these, only proparacaine is approved by the FDA for topical ophthalmic use.[66] After topical application, esters are rapidly absorbed and inactivated in the corneal and plasma esterases. Because of this they do not typically provide adequate levels of intraocular anesthesia.[72] Duration of action with this class of drugs is relatively short, with most reports showing an anesthetic effect of 20-30 minutes in humans and up to 45 minutes in canines.[66,71,80-82]

Cocaine was the first drug found to provide topical corneal anesthesia, but due to its multiple side effects and addictive potential is not readily used today. Proparacaine and

tetracaine have a fairly similar pharmacologic profile and duration of action, although reportedly tetracaine is associated with a more intense burning sensation after application. This is most likely related to its lower pH when compared to proparacaine.[81] Increasing the concentration of the drug applied topically has not been shown to be of benefit, but repeated application of these drugs has been shown to increase the length of anesthesia.[80,82] As these drugs are applied multiple times, the probability of disruption to the corneal epithelial surface increases due to the cytotoxic effects of these drugs to corneal epithelial cells.[83] If the ocular surface is disrupted, penetration of subsequent drugs is accelerated leading to a greater corneal concentration, decreased onset of action, and further risk of toxicity.[66,80]

Antihistamines

Diphenhydramine is an ethanolamine-derivative antihistamine that competitively inhibits histamine at H_1 receptors.[84] The most common use of this drug is in the treatment of allergies, but others uses include as a prevention of motion sickness, an antiemetic, an antitussive, and as a sedative. Diphenhydramine has also been well shown to provide local cutaneous anesthesia in circumstances when allergies to other anesthetics exist.[85-87] While not known definitively, the anesthetic action is believed to be through stabilization of the nerve cell membrane and prevention of depolarization, similar to other local anesthetics.[86] Once applied to the corneal surface in a 5% solution, corneal sensitivity was found to be significantly decreased from baseline through the 90 minute post application recording but did not provide absolute corneal anesthesia in rabbits.[84] This drug may represent a safe alternative to those patients who have a known sensitivity to other topical anesthetics or require long term administration.

Regional Anesthesia

In ophthalmology, anesthesia and akinesia can be provided for diagnostic and therapeutic use by local infiltration of drugs into the periocular or retrobulbar regions using a needle. This administration route allows for inhibition of nerve impulse propagation for variable periods of time depending on the molecular components of the agent used.[67] Typically the duration of blockade provided is longer than that noted after topical application. If injected into the retrobulbar space, vision will be temporarily compromised as the drug is deposited inside the muscular cone and next to the optic nerve.[66] Periocular administration involves placement of the anesthetic around the globe to transiently block sensory nerves from conducting noxious stimuli to the brain. This type of block can be used on the eyelids, subconjunctival tissues, or extraocular muscles and should not affect vision as the drug is not placed near the optic nerve.[66] Regardless of the route of administration chosen, care must be exercised to avoid exceeding the toxic dose for each specific individual.[65,67,71,88]

Topical Ophthalmic Use

The use of topical anesthetics is the most common form of ocular anesthesia used in clinical situations.[66] Advantages of topical administration over ophthalmic regional anesthesia include faster post-operational recovery, lower cost, minimal pain during administration, greater patient satisfaction, and elimination of needle related complications (retrobulbar hemorrhage, ocular perforation, optic nerve damage).[72]

For an ophthalmic drug to penetrate the intact corneal epithelium and stroma, it must be to some degree soluble in both lipid and water.[89] Most drugs prepared for topical use are weak bases and are present in both the ionized and non-ionized forms. These exist in an equilibrium called the free-base form of the drug when buffered to approach the pK_a. Penetration of the

ionized molecules into the cornea depends on the partition theory as they are hydrophilic in the ionized form and hydrophobic in the non-ionized.[89,90] After application to the corneal surface, the non-ionized molecules rapidly penetrate the corneal epithelium and exert their action upon the subbasal nerves.[91] The acidic nature of drugs used topically allows them to penetrate the cornea and sclera. However, this same property provides an uncomfortable burning sensation in most individuals after topical application.[66,71,81] A rapid onset makes them ideal for use in the clinical setting for diagnostic and therapeutic purposes (intraocular pressure assessment, obtaining culture and cytology samples, removal of superficial foreign bodies, keratectomies) as well as facilitating examination of painful corneal conditions. Repeated, chronic application is not recommended because these drugs have been shown to impede corneal epithelial regeneration by blocking epithelial cell mitosis and cell migration.[66,71]

Toxicities with Topical Use

Topical anesthetics are used frequently in ophthalmology for both diagnostic and therapeutic procedures and are considered safe for use in most patients.[79] Problems with use can arise in patients sensitive to the drug and those using or abusing the drug chronically. It is not surprising that a majority of the common toxicities are noted on the ocular surface as this is where they are applied. The most common adverse effect noted with use is a burning or stinging sensation after application.[66,67,71,73,79,81] Superficial punctate corneal epithelial erosions are also noted with some frequency after routine clinical use. The exact cause of the lesions is not known but they may be exacerbated by corneal drying due to lack of sensation and a decreased blink rate.[73,79] Also noted is damage to microvilli of corneal epithelial cells and a decrease in healing rate of epithelial defects due to inhibition of cell migration.[66,73,88] When used chronically for pain relief, healing of corneal defects can be significantly delayed with accumulation of a yellowish-

white stromal infiltrate in circular ring patterns.[92] The rings may represent antigen-antibody complexes similar to those seen in herpetic keratitis.[93] With continued use and damage to the epithelial barrier, patients are predisposed to infectious keratitis and cytotoxic effects on keratocytes.[79,83] Reports also exist of thinning and perforation of corneas with repeated use, even with dilute concentrations of the agents.[94,95] Proparacaine may rarely cause an acute, severe, immediate hypersensitivity that leads to a diffuse keratitis in humans after application. The appearance is that of a gray, ground glass corneal surface which sloughs large sections of necrotic epithelium.[66] Tetracaine also has been reported to cause hypersensitivity reactions locally after application but may be linked to the preservative rather than the drug.[66,79,94]

Systemic side effects are less likely to develop after topical application but have been reported. These include eyelid numbness, headache, anxiety, shortness of breath, and even seizures.[66,79,96] Patients may describe a sudden difficulty in breathing, become drowsy, complain of sudden weakness or fatigue, have unusual pallor, or develop an irregular heartbeat.[66] In these instances it is thought that after topical application in sensitive individuals, absorption occurs rapidly through conjunctival capillaries and enters the blood stream and central nervous system.[79] A fairly uncommon but possible effect is contact dermatitis on skin that the drugs come in contact with chronically, including the fingertips of practitioners.[79,97]

Chapter 2 - Comparison of Efficacy and Duration of Topical Anesthetics on Corneal Sensitivity in Clinically Normal Horses

Introduction

The cornea is one of the most highly innervated structures in the body deriving its sensory nerve supply from the ophthalmic branch of the trigeminal nerve.[8] As the trigeminal nerve branches it gives rise to the long posterior ciliary nerves that penetrate the corneal stroma at the limbus. Despite few nerves present in the deep cornea, the epithelial cell layers and anterior stroma are richly innervated, with unsheathed nerve endings present in the epithelium.[8] The density and distribution of corneal nerves has been extensively studied in humans, dogs, cats, and rabbits but has not been determined to date in horses.[15,98,99]

Corneal sensitivity was first documented by use of an aesthesiometer in 1894 by Frey.[23] Since then many different aesthesiometers have been developed, including the Cochet-Bonnet corneal aesthesiometer in 1960. The Cochet-Bonnet has become the most commonly used aesthesiometer in both clinical and research settings.[23] This device uses a 0.12 mm nylon monofilament of varying exposed lengths from 6.0 to 0.5 cm. These lengths can be converted to pressure readings when applied to the cornea of 11 to 200 mg per 0.0113 mm^2. A longer filament length applies less pressure to the corneal surface, whereas a shorter filament is more rigid and provides 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 (CTT) is the minimal amount of corneal stimulation that results in a blink reflex and is measured by use of a corneal aesthesiometer.[29] The CTT has been studied

as a measure of corneal sensitivity in horses of varying ages and breeds and its mean value has been reported to range from 2.12 ± 0.62 to 5.01 ± 0.61 cm when using the Cochet-Bonnet corneal aesthesiometer.[28,49,100,101] Previous studies have shown the central portion of the equine cornea to be the most sensitive, followed by the nasal, temporal, ventral, and dorsal regions.[49,100] It is presumed that the differences in sensitivity by location are related to the nerve fiber density in these locations, as the central portion of the cornea in other animals has the greatest corneal nerve fiber density.[12,22,102,103]

Topical corneal anesthesia is frequently utilized in both human and veterinary ophthalmology for a variety of diagnostic procedures including measurement of intraocular pressure, corneal or conjunctival cytological sampling, facilitating examination of painful corneal diseases, anterior chamber paracentesis, and removal of ocular foreign bodies. While various anesthetics are used for corneal anesthesia, one of the most commonly utilized and studied topical anesthetics in veterinary ophthalmology is 0.5% proparacaine. This drug has a rapid onset of less than 5 minutes in most species with a varied duration, lasting 25 minutes in horses and cats and 45 minutes in dogs.[28,80,104] The manufacturer recommends keeping the drug refrigerated when not in use and it has been shown to have decreased efficacy if not maintained accordingly.[105] The storage requirement of proparacaine makes this drug difficult to maintain in certain clinical situations and when traveling to evaluate patients in the field. Other anesthetic drugs which do not require refrigeration have been evaluated for topical ophthalmic use in humans and various species including bupivacaine, lidocaine, tetracaine, and mepivacaine[71,72,76,78,101,106] but the comparative efficacy of these local anesthetics in the equine eye has not been reported.

The purpose of this study was to compare the efficacy and duration of various topical anesthetics on corneal sensitivity in clinically normal horses as determined by CTT measured with a Cochet-Bonnet corneal aesthesiometer.[a] Drugs evaluated included 0.5% proparacaine,[b] 0.5% bupivacaine,[c] 2% lidocaine,[d] and 2% mepivacaine.[e]

Materials and Methods

Animals

This study utilized 60 Quarter horses from the Kansas State University Animal Science & Industry Horse Unit and 8 privately owned horses of varying breeds. The study group consisted of 24 geldings and 44 mares ranging in age from 2 to 20 years (mean 6.3 years). Breeds represented included Quarter horses (64), Arabian/Arabian-cross (2), and one each of American Saddlebred and Pony. All horses underwent an ophthalmic examination consisting of slit lamp biomicroscopy examination,[f] Schirmer tear test I,[g] and rebound tonometry.[h] Only animals deemed to be clinically normal and having a Schirmer tear test > 10 mm/min were included. The study was performed using gentle manual restraint 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[f] was used to measure CTT in one randomly selected eye of each horse (32 right eyes, 36 left eyes). The eye chosen was selected by flipping a coin. The aesthesiometer contains a 0.12 mm diameter nylon filament of adjustable length (6.0 - 0.5 cm) that was gently applied perpendicular to the central portion of the cornea to determine sensitivity. Pressure was increased until a small deflection of the filament was noted, approximating a 4% deflection. Aesthesiometer filament readings may be converted to applied force measurements

using a conversion chart provided by the manufacturer. Readings are then displayed as pressure readings in either grams per square millimeter or milligrams per S (S= 0.0113 mm^2 of sectional area of the filament).

A baseline CTT was obtained on all horses prior to administration of a topical anesthetic medication. The baseline CTT was determined by applying the filament to the central portion of the cornea at maximal length (6 cm) and assessing for a blink response. If no response was noted, the filament was decreased in length by 0.5 cm increments and applied again until a blink reflex was noted to occur on at least 3 out of 5 stimulations (**Figures 1.3-1.5**). The length of the filament which stimulated a reproducible blink response was recorded as the CTT measurement in cm. Each horse was then administered one of the drugs topically and measurements taken over 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 (i.e. shortest filament length, 0.5 cm) indicating complete corneal anesthesia. The same investigator (JDP) performed all measurements, thereby minimizing a confounding variable of inter-observer variance. Care was taken to avoid manipulation of eyelids and stimulation of vibrissae. Ambient temperature was recorded in degrees Fahrenheit for each day testing was performed (range 42-95°F).

Treatment

The study population consisted of two separate groups, with each group subjected to a different experimental design. Horses in group 1 were subjected to a completely randomized design (CRD) whereby each horse was randomly assigned to receive 0.5% proparacaine,[b] 0.5% bupivacaine,[c] 2% lidocaine,[d] or 2% mepivacaine,[e] with the eye to be treated also selected at random. Privately owned horses were placed in group 2, with each horse receiving all 4 treatments in a replicated Latin square design with a one week washout period between

treatments. For these 8 horses, the eye selected at random prior to application of the first drug was maintained throughout the data collection time points. After determination of baseline CTT, 0.2 mls of the selected drug was drawn into a 1 ml syringe. The 27 gauge needle was broken off at the hub and the drug was gently sprayed onto the dorsal corneal surface. The same bottle of each drug was used throughout the study period and stored per the manufacturer's guidelines. The time of administration of topical anesthetic was designated time 0 (T0). Any adverse reaction to application of the drugs was monitored for at this time. Corneal touch threshold was then measured 1 minute following application of the drug (T1), and every 5 minutes until 60 minutes post-treatment (T5-T60). After this time point, CTT measurements were taken every 10 minutes until 90 minutes post-treatment (T70-T90). Each reading after the application of the chosen drug was initiated at a length of 1.5 cm longer than the last stimulation and decreased in 0.5 cm increments until a consistent blink response was noted with 3 out of 5 stimulations. After completion of the final measurement both corneas were stained with fluorescein[i] and examined with a slit lamp to ensure intact corneal epithelium.

Data Analysis

Data from each group was analyzed separately in order to reflect the different underlying experimental designs. In both cases, a general linear mixed model was fitted to the response "corneal touch threshold" (CTT) assuming a Gaussian distribution. The models included the fixed effects of drug treatment (0.5% proparacaine, 0.5% bupivacaine, 2% lidocaine or 2% mepivacaine), time (T0-T90) and their 2 way interaction. The fixed effect of sex, as well as the covariates age and ambient temperature, were evaluated for inclusion in the model based on their *P*-values. In the statistical model fitted to data from group 1 (CRD), a first-order antedependence structure was used to model the residual variance-covariance matrix in order to accommodate

heterogenous 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), the random blocking factors of horse and period were included. A spatial power variance-covariance structure was fitted to the residuals in order to account for repeated observations on a given horse-period-drug combination over time. All variance-covariance structures were selected based on a model fit using Bayesian Information Criterion.

Kenward and Roger's approach was used for computing degrees of freedom and estimating standard errors. The models were fitted using the GLIMMIX procedure of SAS.[j] Model assumptions were evaluated using studentized residual plots and assumptions were considered to be appropriately met. Pairwise comparisons were conducted using Bonferroni's adjustment to avoid inflation of Type I error rate. A value of $P < 0.05$ was considered significant.

Results

At baseline, there was no evidence for differences in mean CTT values between any of the treatment groups. For all 4 drugs evaluated in this study the onset of action was rapid, with maximal effect noted by the 5 minute post-application reading (T5). All horses returned to baseline CTT by 90 minutes post application, regardless of the specific topical anesthetic. Evidence for a significant drug by time interaction on CTT ($P<0.0001$) was noted in both groups, thus indicating that the dynamics of corneal sensitivity over time differed between local anesthetics. Figures 1 and 2 illustrate the estimated least square mean CTT for each drug over time for the CRD and replicated Latin square designs, respectively. No adverse effects after application were noted in any animal during the period of the study.

There was no evidence for any association of ambient temperatures, as recorded on testing days, and CTT measurements. Also, there was no evidence for an effect of sex on CTT

detected in this study. However, a significant positive effect of age on CTT (P= 0.0087) was apparent, whereby each one year increase in age was associated with an estimated increase of 0.02 ± 0.01 cm of CTT regardless of treatment.

Group 1 (Completely Randomized Design)

Least square mean (± SE) baseline CTT results were not significantly different between treatments and ranged from 2.96 ± 0.14 to 3.04 ± 0.14 cm. Relative to baseline, corneal sensitivity dropped sharply by 1 minute after application for all 4 drugs (P< 0.0001) and reached a minimum by 5 minutes post application. The minimum estimated CTT values were 0.01 (± 0.06), 0.00 (± 0.06), 0.03 (± 0.06), and 0.4 (± 0.06) cm for proparacaine, bupivacaine, lidocaine, and mepivacaine respectively, and were apparent by 5 minutes after application. The effect and duration of the topical anesthetics was assessed by comparing differences in CTT over time relative to baseline for each drug. Corneal sensitivity was significantly decreased relative to baseline through 35 minutes after application for proparacaine (P< 0.03) and mepivacaine (P< 0.0001), 45 minutes after application for lidocaine (P< 0.0001), and 60 minutes after application for bupivacaine (P< 0.03) (**Table 2.1 and Figure 2.1**).

Group 2 (Replicated Latin Square)

Least square mean (± SE) baseline CTT results were not significantly different between treatments and ranged from 3.65 ± 0.21 to 3.88 ± 0.21 cm. Relative to baseline, corneal sensitivity decreased rapidly by 1 minute after application for all 4 drugs (P< 0.0001) and reached a minimum by 5 minutes post application. The minimum estimated CTT for each drug was 0.03 (±0.21), 0.02 (± 0.21), 0.25 (± 0.22), and 0.82 (± 0.21) cm for proparacaine, bupivacaine, lidocaine, and mepivacaine respectively and were apparent by 5 minutes after application. The duration of effect was assessed by comparing differences in CTT over time

relative to baseline for each drug. Corneal sensitivity was significantly decreased relative to baseline through 35 minutes after application for proparacaine (P< 0.001), 40 minutes for mepivacaine (P< 0.004), 55 minutes after application for lidocaine (P< 0.04), and 60 minutes after application for bupivacaine (P< 0.02) (**Table 2.1 and Figure 2.2**).

Table 2.1 Least square mean CTT results for the Completely Randomized Design (CRD) and Replicated Latin Square. Maximal anesthetic effect (MAE) represents the recording times at which the maximal effect was noted. Duration reported is the length of time through which the effect was significantly different from baseline CTT.

Drug	Baseline CTT ± SE (cm)	Lowest CTT (cm)	MAE (min)	Duration (min)
CRD (Group 1)				
0.5% proparacaine	2.96 ± 0.14	0.01	T5	T35
2% lidocaine	3.39 ± 0.14	0.03	T5	T45
0.5% bupivacaine	3.04 ± 0.14	0.01	T5-T15	T60
2% mepivacaine	3.28 ± 0.14	0.06	T5-T10	T35
Replicated Latin Square (Group 2)				
0.5% proparacaine	3.65 ± 0.22	0.03	T5-T10	T35
2% lidocaine	3.81 ± 0.22	0.22	T5-T10	T55
0.5% bupivacaine	3.80 ± 0.21	0.02	T5-T10	T60
2% mepivacaine	3.88 ± 0.21	0.82	T5-T10	T40

Discussion

Results of our study indicate that topical application of all four drugs evaluated decreased corneal sensitivity in clinically normal horses. The mean baseline corneal sensitivity did not vary

significantly between drug treatments within a given group and had a range of 2.9 to 3.9 cm, which is in accordance with previous equine studies using the same aesthesiometer.[28,49,100,101,107] Statistically significant differences from baseline were noted over time with each drug and also between drugs at various time points after drug administration. All drugs evaluated began to exert their effect of corneal desensitization within the first minute, with the maximal effect noted by the 5 minute reading. The duration of effect on corneal sensitivity was varied between drugs with bupivacaine providing the longest acting effect.

In contrast to a recent report,[28] we found proparacaine to decrease corneal sensitivity to a level approaching complete anesthesia and remain significantly decreased relative to baseline through 35 minutes post application. Difference in the corneal anesthetic effects of proparacaine between studies could be breed related as the previous study evaluated adult Thoroughbreds whereas we used mostly Quarter horses. Significant differences in CTT have been noted between different facial conformations in both canine and feline patients with brachycephalic animals having the lowest corneal sensitivity.[12,108] Similar differences may exist between breeds of horses but have not been documented and due to the small sample size of other breeds represented in this study, a formal comparison between breeds could not be conducted. The anesthetic duration reported here is also longer than previously reported in horses and cats[28,104], but shorter than in dogs.[80] However, it may be inappropriate to compare anesthetic duration between species as this study applied a larger volume of drug to the corneal surface than the single drop used in cats and dogs.

The application of 2% lidocaine to the corneal surface appears to provide a comparable efficacy and duration of anesthesia to that of proparacaine in clinically normal eyes. Lidocaine has been used empirically in clinical practice to desensitize horse corneas, but to our knowledge,

this is the first study to evaluate its effect and efficacy. The formulation of lidocaine used in this study is more commonly utilized in veterinary medicine for local nerve blockade and treatment of ventricular arrhythmias. In human ophthalmology, lidocaine can be used for many diagnostic and therapeutic procedures of the cornea and anterior segment, including phacoemulsification.[72,76,109,110] The lack of refrigeration requirements and apparently comparable activity to proparacaine make lidocaine a potential alternative for providing corneal desensitization in horses. The use of a topical ophthalmic lidocaine gel formulation was not evaluated in this study and so the effects in equine patients cannot be stated.

In human ophthalmology, bupivacaine is used for topical, subconjunctival, retrobulbar, and intracameral anesthesia with adequate effect.[66,67,71,72] There have previously been no reported studies evaluating use of the injectable formulation of bupivacaine for topical corneal anesthesia in equines. Prolonged corneal anesthetic activity, relative to the other drugs evaluated, was noted after application of 0.5% bupivacaine in these horses. This is in accordance with the prolonged effect noted when used in other regions of the body.[65,74] The increased duration of action of bupivacaine is reported to be related to its high degree of protein binding and lipid solubility.[66,67,71] This increased duration could be beneficial when performing procedures in 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 intracamerally in humans during cataract surgery or other ocular procedures and provides adequate desensitization.[70,111] Although there was a reduction noted in corneal sensitivity with topical application of mepivacaine, this drug did not reach the same anesthetic effect as the other drugs in this study. The reduced efficacy of mepivacaine is most likely related to its poor corneal permeability, as was noted previously in

rabbits.[78] Based on the results of this study, we do not recommend using 2% mepivacaine for topical ophthalmic anesthesia in horses seeing that it does not produce the same degree of effect as other drugs.

Amide or ester-based anesthetic drugs, such as those investigated, exert their activity by penetrating the corneal surface and inducing a reversible nerve conduction block after being applied topically 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.[66,71] This block is thought to take place by decreasing the permeability of sodium and other ions, thereby inhibiting depolarization of the cell membrane and halting propagation of pain sensations.[60,67] The rapid onset of action with the drugs used is likely due to their high lipid solubility, which allows for penetration of cell membranes and rapid presentation to the target receptors.[60,66,67] The pH of the drug also plays a role in the degree of block provided as only the non-ionized portion of the drug is available to bind to the receptors. The closer the pH is to the pKa for a selected drug, the greater the number of non-ionized particles that are present.

Previous reports in humans have proposed alterations in corneal sensitivity to be associated with hormonal status, sex, age, ambient temperature, humidity, and ambient lighting.[23,51] In our study, we attempted to minimize these factors by having the same investigator (JDP) perform all testing, by using the same materials, and by testing in similar environments. Our methods appeared to be adequate as all horses returned to their baseline CTT values by the end of the manipulations, and in group 2 horses the baseline CTT values between study periods were numerically very similar. In our study, we were not able to detect differences in CTT as a function of ambient temperatures or sex. We were able to determine a significant difference in CTT with increasing age as has also been shown previously in humans.[23] However,

the estimated magnitude of the association between age and decreased corneal sensitivity, while statistically significant, is not likely to be clinically relevant.

Potential limitations of this study are that the investigators were not masked to the drugs applied and corneal epithelial toxicity was not evaluated. An attempt to prevent potential bias was made by randomly assigning horses to drug treatments in group 1 and by enrolling horses in the group 2 design to evaluate the effect of each drug within the same individual. No overt adverse clinical effects were noted after application of the drugs at any time point, but without a microscopic examination of the corneal epithelial cells the safety of topical anesthetic application cannot be definitively ascertained. However, in human ophthalmology these drugs are viewed as generally safe when used in a controlled clinical setting.[79]

Lastly, this study was performed on clinically normal horses and so it is not known how these drugs would perform in cases of corneal disease. A previous report found that in diseased states, the pH of the corneal surface is lowered, leading to increases in blood flow of corneal blood vessels and reducing the efficacy of the agents through dilution.[112] Future studies need to be conducted in horses with various corneal pathologies to determine the effect on drug efficacy and duration.

Based on the results of this study, 0.2 mls of 0.5% proparacaine or 2% lidocaine applied topically appears to provide a marked reduction in corneal sensitivity in clinically normal horses and should be of benefit for procedures lasting a short duration. Bupivacaine is recommended for procedures in which a longer duration of decreased corneal sensitivity is needed. Although mepivacaine significantly decreased corneal sensitivity as compared to baseline CTT, the topical use of this drug is not recommended as the level of desensitization does not reach the same magnitude as that with proparacaine, lidocaine, and bupivacaine. Regardless of the topical

anesthetic applied, it is important to provide lubrication to the corneal surface during periods of corneal anesthesia as lacrimation stimulation and the blink reflex may be temporarily reduced.

Footnotes

[a.] Cochet-Bonnet aesthesiometer, Luneau Ophtalmologie, Chartres Cedex, France.

[b.] 0.5% Proparacaine, Alcon laboratories Inc., Fort Worth, TX, USA

[c.] 0.5% Bupivacaine, Hospira Inc., Lake Forest, IL, USA

[d.] 2% Lidocaine, Hospira Inc., Lake Forest, IL, USA

[e.] 2% Mepivacaine, Pfizer Inc., New York, NY, USA

[f.] SL-15®, Kowa Company, Ltd, Tokyo, Japan

[g.] Schirmer Tear Test, Schering-Plough Animal Health, Union, NJ, USA

[h.] TonoVet®, Jorgensen Laboratories, Loveland, CO, USA

[i.] Bio Glo®, HUB Pharmaceuticals LLC, Rancho Cucamonga, CA, USA

[j.] SAS Version 9.2, SAS Institute Inc., Cary, NC, USA

Figure legends

Figure 2.1. Estimated least square mean CTT over time after application of bupivacaine, lidocaine, mepivacaine, and proparacaine in horses subjected to a completely randomized design. Rapid onset of action is noted with maximal effect evident by 5 minutes.

Figure 2.2. Estimated least square mean CTT over time after application of bupivacaine, lidocaine, mepivacaine, and proparacaine in horses subjected to a replicated Latin square design. Rapid onset of action is noted with maximal effect evident by 5 minutes.

Figure 2.1 Completely Randomized Design

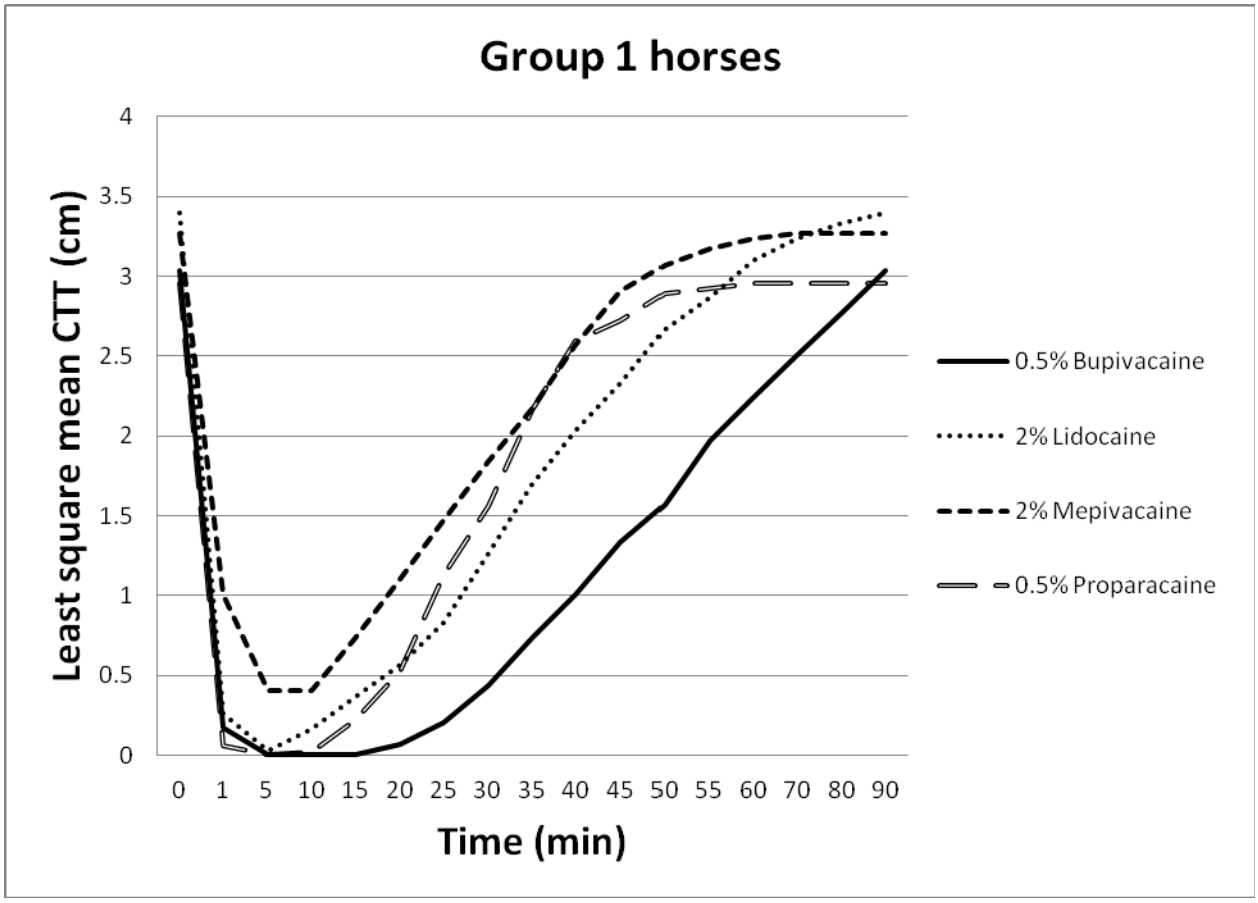
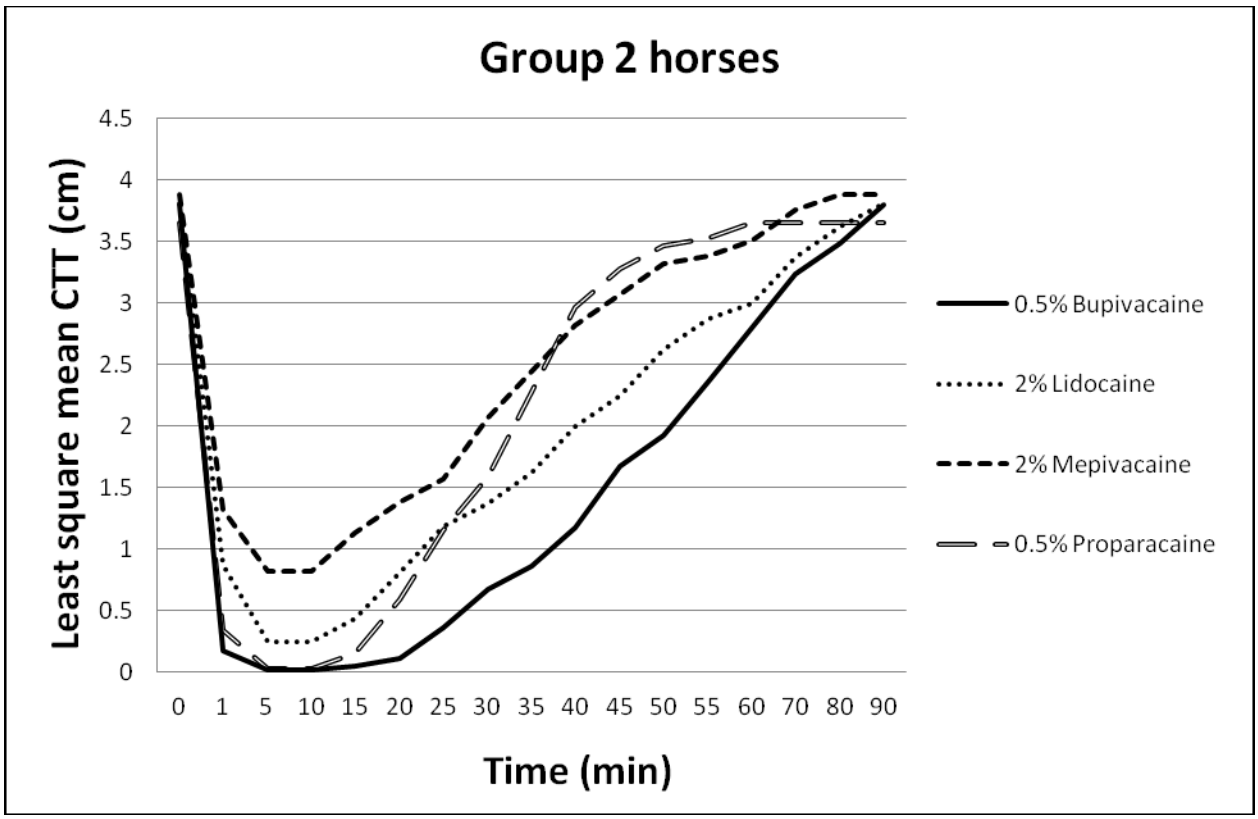


Figure 2.2 Replicated Latin Square


Group 2 horses
Least square mean CTT (cm)
4.5
4
3.5
3
2.5
2
1.5
1
0.5
0
0 1 5 10 15 20 25 30 35 40 45 50 55 60 70 80 90
Time (min)
0.5% Bupivacaine
2% Lidocaine
2% Mepivacaine
0.5% Proparacaine

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