Evaluation of the effects of age and pituitary pars intermedia dysfunction on corneal sensitivity in horses

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Objective—To determine effects of age and pituitary pars intermedia dysfunction (PPID) on corneal sensitivity in horses.

Animals—20 adult horses allocated into 3 groups (PPID group, old [> 15 years old] horses with PPID \( n = 5 \); old group, old (> 15 years old) horses without PPID \( n = 9 \); and young group, young [≤ 10 years old] horses without PPID \( n = 6 \)). All horses with PPID had hirsutism and abnormal fat deposition or laminitis; none of the old or young horses had hirsutism, abnormal fat deposition, or laminitis.

Procedures—A Cochet-Bonnet aesthesiometer was used to measure the corneal touch threshold (CTT) in both eyes of each horse. The nylon monofilament was applied at a maximum length of 60 mm to the central region of the cornea and length was decreased by 5-mm increments until a consistent blink response was elicited. Tear production was assessed in all eyes via the Shirmer tear test (STT).

Results—Mean ± SD CTT was significantly greater for young horses (47.50 ± 4.52 mm) than for horses in the old (28.06 ± 5.72 mm) and PPID (21.5 ± 3.37 mm) groups. Old horses had significantly higher CTT values than did horses with PPID. The STT values were within the reference range for all groups and did not differ significantly among groups.

Conclusions and Clinical Relevance—Corneal sensitivity decreased with both age and PPID. Because decreased corneal sensitivity is associated with impaired wound healing, increasing age and PPID may increase the risk for nonhealing or recurrent corneal ulcers in horses. (Am J Vet Res 2013;74:1030–1035)

The cornea is the most densely innervated tissue of the body.1 Most of the corneal nerve fibers are sensory, originating from the ophthalmic branch of the trigeminal nerve.2 Nerves enter the cornea at the anterior stroma and branch toward the epithelium, which is densely innervated by unmyelinated nerve endings.3 Keratocytes are located in close proximity to nerve fibers and will occasionally envelop adjacent nerve fibers in cytoplasmic extensions.3 This close association is important because nerve fibers exert trophic influences on the corneal epithelium and serve to maintain a healthy ocular surface.3,4 Neurons and epithelial cells provide support to each other through the mutual release of neurotransmitters and neuropeptides, which stimulate growth, proliferation, and differentiation of corneal epithelial cells and production of type VII collagen.5,6

In humans, dysfunction of corneal innervation induces a degenerative condition of the corneal epithelium and stroma known as neurotrophic keratopathy.1,6 In patients with neurotrophic keratopathy, reduced corneal sensitivity can result in recurrent or chronic epithelial defects that may lead to subsequent ulceration and perforation. Reduced corneal sensory innervation results in a decrease in the vitality, metabolism, and mitotic activity of epithelial cells, which may be responsible for the epithelial breakdown and corneal ulceration observed in these patients.9

Systemic disease in veterinary and human patients can negatively affect corneal innervation, which can result in decreased corneal sensitivity and the development of neurotrophic keratopathy. In human patients, neurotrophic keratopathy has been associated most frequently with long-standing diabetes mellitus, herpes zoster ophthalmicus, herpes simplex keratitis, and brainstem neurologic disease or trauma.10 A correlation between corneal sensitivity and systemic illness has been identified in neonatal foals.11 Corneal sensitivity is reduced in dogs with diabetes mellitus.12 Corneal sensitivity is reduced in approximately 20% of human patients with diabetes mellitus.13-18
degree of sensory neuropathy and retinopathy, age, and duration of the disease, which has led to the use of in vivo confocal microscopy for the measurement of corneal nerve fiber density and to monitor disease progression. Decreased corneal sensitivity and impaired neural regulation in the cornea or in diabetics also leads to problems with epithelial wound healing and recurrent erosions. Pituitary pars intermedia dysfunction, a common endocrinopathy of older horses associated with peripheral neuropathy and impaired wound healing, may also adversely affect corneal neurons and play a role in impaired corneal healing.

Pituitary pars intermedia dysfunction, commonly referred to as equine Cushing’s syndrome, is the most common endocrinopathy of older horses and ponies. Clinical, pharmacological, biochemical, and histologic data indicate that PPID is a neurodegenerative disease with loss of dopaminergic inhibitory input to the melanotropes of the pars intermedia. Similar to other neurodegenerative diseases, age is the most important risk factor for the development of PPID. Clinical signs are typically detected in horses that are 18 to 20 years old, with only rare reports in horses <10 years old. Clinical signs of PPID include hirsutism, laminitis, bulging supraorbital fat, abnormal fat distribution, lethargy, polyuria and polydipsia, susceptibility to infections, hyperhidrosis, and inappropriate lactation. Treatment with pergolide, a dopamine-receptor agonist, results in improvement of both the clinical signs and biochemical abnormalities associated with the disease. In a recent large retrospective case series of horses and ponies, 50% of the patients with PPID were alive 4.6 years after diagnosis and 25% were alive at 5.3 years after diagnosis. In that study, the cause of death for 73% of the horses and ponies was euthanasia because of 1 or more clinical signs typically associated with PPID.

Deficits in corneal innervation result in reduced corneal sensitivity. This reduction can be quantified with corneal aesthesiometers, which are used to evaluate corneal sensitivity by measuring the CTT. The CTT is reported as the millimeter length of the aesthesiometer filament or as g/mm², a unit of pressure derived from filament length. When the threshold pressure to stimulate corneal sensory nerve endings has been reached, the corneal reflex is induced, which results in a blink, retraction of the globe, and elevation of the third eyelid. A higher CTT value corresponds to a higher degree of corneal sensitivity.

The Cochet-Bonnet aesthesiometer has been used to assess corneal sensitivity in many veterinary species. Corneal sensitivity in healthy adult horses has been reported. In a study of corneal sensitivity in a group of Warmblood horses, the mean CTT was 19.7 mm. In 3 studies of corneal sensitivity that involved predominantly Thoroughbreds, the CTT value in healthy adult horses was 48.0 mm, 48.0 mm, and 37.9 mm. The effect of age on corneal sensitivity in horses was evaluated in 2 studies. In 1 study that involved predominantly Thoroughbreds, healthy neonatal foals had a higher CTT (50.1 mm) and corresponding higher corneal sensitivity, compared with the CTT in healthy adult horses (48.2 mm); however, these values did not differ significantly. In another study conducted to evaluate corneal sensitivity in 50 healthy adult Warmblood horses categorized into 3 age groups (1 to 4, 5 to 10, and >10 years old), there was a nonsignificant decrease in corneal sensitivity with age in the nasal, temporal, ventral, and central region of the cornea and a nonsignificant increase with age in the dorsal region of the cornea.

Retrospective studies have identified increasing age as a risk factor for development of refractory corneal ulcers in multiple species of animals, including horses, dogs, and cats. A reduction in corneal innervation with decreased corneal sensitivity may play a role in this syndrome. Corneal denervation substantially impairs the healing ability of corneal epithelium after corneal injuries and predisposes corneas to recurrent, spontaneous epithelial erosions.

The relationship among corneal sensitivity, PPID, and age in horses is currently unknown. Therefore, the purpose of the study reported here was to determine the effects of age and PPID on corneal sensitivity in horses.

Materials and Methods

Animals—Client-owned clinically normal horses that were examined by a board-certified veterinary ophthalmologist (MLU) and found to be free of ocular disease (except for small capsular or cortical cataracts and nuclear sclerosis) were used in the study. Informed consent was obtained from all owners for inclusion of the horses in the study. The University of Pennsylvania Animal Care and Use Committee approved the protocol and procedures used in the study.

An ideal study for the evaluation of the effects of age and PPID status would have been a 2 X 2 design, with changes for both factors independently in subjects. In a recent study, horses with PPID typically were >15 years old, with 7 (3%) PPID horses <10 years old, 35 (16%) PPID horses 10 to 15 years old, and 175 (81%) PPID horses >15 years old; mean age of horses at the time of diagnosis of PPID was 21.6 years. Because young horses rarely develop PPID, constructing a 2 X 2 design with age and PPID status as independent variables was not possible. Therefore, horses were allocated into 3 groups on the basis of age and PPID status. The young group comprised horses ≤10 years old that did not have PPID; thus, neither age nor PPID was likely to be associated with effects on corneal sensitivity. The old group comprised horses >15 years old that did not have PPID; thus, age but not PPID was likely to be associated with effects on corneal sensitivity. The PPID group comprised horses >15 years old that had PPID; thus, both age and PPID were likely to be associated with effects on corneal sensitivity.

All horses with PPID had hirsutism (ie, abnormal coat and shedding), which is associated with a positive predictive value of 90% for PPID (compared with postmortem examination as the criterion-referenced standard), as well as abnormal fat deposition or laminitis. Horses that did not have PPID were assigned to the young or old groups on the basis of ages of horses with PPID reported in a recent study.
STT—The STT was conducted with a 35 X 5-mm commercial test stripa in both eyes of each horse. The STT was conducted for all horses in a similar environment and at a similar time of day, time of year, and ambient temperature to minimize effects of those variables on tear production. Tear production was recorded as millimeters of wetting per minute.

CTT—The CTT was measured with a Cochet-Bonnet aesthesiometerb in a quiet environment while the horses were restrained in a standing position without the use of sedation or application of auriculopalpebral nerve blocks. In both eyes of each horse, the 0.12-mm-diameter nylon monofilament of the aesthesiometer was placed in contact with the central region of the cornea. Pressure applied to the cornea was altered by changing the length of the nylon monofilament, which could be adjusted to lengths of 5 to 60 mm. The filament was initially applied at a maximum length of 60 mm. When a consistent blink response was not elicited at that length, the filament length was decreased (increments of 5 mm) and retested until a consistent blink response was elicited. For each eye, filament length that elicited a blink response for 3 consecutive assessments was recorded as the CTT. Standard instrument curves allowed values to be reported as nylon filament length (scale, 6.0 to 0.5 cm) and 2 mean pressure values (scale, 11 to 200 mg/0.0113 mm² and 0.96 to 17.68 g/mm², respectively). Long monofilament lengths are associated with high CTT values and increases in corneal sensitivity. The same investigator (MLU) performed all measurements to minimize interobserver differences in technique.

Statistical analysis—Distributions for CTT and STT data were tested for normality with a Kolmogorov-Smirnov test.c For normally distributed dependent variables, a mixed-model ANOVA was used to assess difference among group means, with eye (right or left) as a 2-level repeated measure, and group (young, old, or PPID) as a 3-level between-subjects measure. Analyses were performed with commercially available software.d Values of \( P < 0.05 \) were considered significant. The Mann-Whitney U test was used to assess differences among groups for any nonparametric data.e

Results

Animals—Twenty client-owned clinically normal horses (age, 3 to 36 years) were included in the study. There were 11 geldings and 9 mares. The PPID group comprised 5 horses (mean age, 23.2 years; range, 16 to 36 years), the old group comprised 9 horses (mean age, 23.3 years; range, 19 to 31 years), and the young group...
compared 6 horses (mean age, 8.0 years; range, 3 to 10 years). Breeds represented included Warmblood (n = 7), Quarter Horse, (4), Welsh Pony or pony (4), Standardbred (2), Arabian (2), and Appaloosa (1).

STT—The STT values were normally distributed; thus, parametric tests were used to compare differences among means for tear production. There was no significant difference between eyes (right or left) or among groups (young, old, or PPID) for STT values (Figure 1).

CTT—The CTT values were normally distributed; thus, parametric tests were used to compare differences among means for corneal sensitivity. There was no significant difference in CTT between the left and right eyes, and there was not a significant interaction between eye and group. The CTT values differed significantly (P < 0.001) among groups. Mean ± SD CTT was significantly higher for young horses (47.30 ± 4.52 mm) than for both old horses (28.06 ± 5.72 mm) and horses with PPID (21.5 ± 3.37 mm), and old horses had a significantly higher CTT than did horses with PPID (Figure 2). There was no significant difference in mean CTT between geldings and mares. Because of the small sample size, differences in CTT among breeds could not be analyzed.

We performed a linear analysis with age in years as a ratio-scaled dependent variable, rather than age group as a nominally scaled between-subjects variable as in the ANOVA, and CTT in millimeters (mean for both eyes because there was not a significant difference in CTT between the left and right eyes). This analysis revealed a significant (P < 0.001) negative correlation between age and CTT (r = –0.83; r² = 0.686; Figure 3).

Discussion

In the study reported here, corneal sensitivity was significantly reduced in horses 15 years old, both with and without PPID, compared with corneal sensitivity in horses ≤ 10 years old. Within the horses > 15 year old, those with PPID had a significant decrease in corneal sensitivity; compared with the corneal sensitivity for those that did not have clinical signs of PPID. By use of age as a continuous dependent variable, rather than a categorical independent variable, a significant negative correlation was identified between corneal sensitivity and age.

Age affects corneal sensitivity in humans, with children having greater corneal sensitivity than adults. Sensitivities of the peripheral cornea begins to decrease at an earlier age and progresses at a faster rate, compared with the rate of decrease for the central cornea. In 2 studies of corneal sensitivity in horses, corneal sensitivity differed but not significantly among horses of different ages.

A decrease in corneal sensitivity in the presence of systemic disease has been reported in both human and veterinary patients. Compared with age-, skull type-, and sex-matched nondiabetic dogs, diabetic dogs had a significant reduction in corneal sensitivity in all regions of the cornea measured with a Cochet-Bonnet aesthesiometer. Sick neonatal foals admitted to a university hospital had a significant reduction in CTT, compared with the CTT in healthy neonatal foals. A decrease in corneal sensitivity has also been reported in humans with diabetes mellitus. The decrease in corneal sensitivity in humans with diabetes mellitus is believed to be a component of the diffuse neuropathy that affects the peripheral sensorimotor nervous system.

Diabetic neuropathy is characterized by segmental demyelination and axonal degeneration, but the mechanism for the neuropathy is not known. In 1 study, human patients with type 1 diabetes mellitus had a reduction in the number of long nerve fiber bundles in the cornea. Facial palsy, trigeminal neuralgia, optic neuritis, and internal and external ophthalmoplegia have also been detected in humans affected by diabetic polyneuropathy.

In the present study, horses with clinical signs of PPID had significantly lower corneal sensitivity, compared with that for horses without clinical signs consistent with PPID. Similar to other systemic diseases, specifically endocrinopathies in human and veterinary patients, PPID in horses may result in exposure of corneal neurons to circulating factors that leads to corneal neuropathy and a subsequent decrease in corneal sensitivity. Pituitary pars intermedia dysfunction in horses is characterized by increases in plasma and serum concentrations of ACTH, glucose, and insulin; however, concentrations of these analytes may be within the respective reference ranges in some horses with PPID. Plasma cortisol concentration in horses with PPID is variable and may be lower.

In addition to cortisol, other proopiomerocorticoid-derived hormones, including corticotropin-like intermediate peptide, ACTH, melanocyte-stimulating hormone, and β-endorphins, could have glucocorticoid-like activity. One or a combination of these substances may negatively affect corneal neurons in horses with PPID. Further research into the effect of these substances on equine keratocytes and neurons is required to determine the effect of these factors in decreasing corneal sensitivity.

Because of the small sample size in the present study, no conclusions could be drawn with respect to corneal sensitivity and sex in horses. In human patients, males were found to have reduced sensitivity in both the cornea and conjunctiva, compared with that in females, which is consistent with sex differences in other sensory systems (including the skin and olfactory system) and in the general perception of pain.

Studies in horses to identify differences in corneal sensitivity among mares, geldings, and stallions would be important because humans have sex differences in corneal sensitivity to hormonal changes.

The mean CTT for all horses in all 3 groups was 32.25 mm, which is comparable to the mean CTT reported in other studies in which investigators examined corneal sensitivity in adult horses (primarily Thoroughbreds in the United States). Those investigators reported CTT values in healthy adult horses of 48.0 mm, 48.0 mm, and 37.9 mm. In contrast, a mean CTT of 21.15 mm was reported for primarily
Warmblood-type horses in Switzerland. Possible reasons for this difference could be behavioral or training differences among populations of horses, breed differences in corneal sensitivity, differences in application of the Cochet-Bonnet aesthesiometer by the investigators, or a combination of these factors. Ambient temperature and relative humidity can affect the nylon filament of the Cochet-Bonnet aesthesiometer and alter the stimulus applied, which also could have accounted for the difference in CTT among studies.

A potential limitation of the present study was the difficulty in obtaining aesthesiometer measurements for the cornea of adult horses. Evaluating the blink response after application of the Cochet-Bonnet aesthesiometer filament is subjective, and the potential for stimulating a blink response by touching the cilia, rather than the cornea, is substantial; however, these potentially confounding factors were minimized by having the same investigator perform all measurements with similar restraint and in a similar stall environment for each horse.

In the present study, corneal sensitivity decreased with increasing age of horses and with PPID. These findings may explain why older horses with PPID have anecdotally been described as having abnormal corneal findings may explain why older horses with PPID have anecdotally been described as having abnormal corneal findings and, ultimately, the incidence, healing rate, and recurrence rate of corneal ulcers in horses with PPID.

References

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