Objective evaluation of the systemic effects of topical application of 1% atropine sulfate ophthalmic solution in healthy horses

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OBJECTIVE
To determine the safety of topical administration of 1% atropine ophthalmic solution in healthy horses by objectively measuring gastrointestinal transit time.

DESIGN
Randomized, masked, controlled crossover study.

ANIMALS
6 adult geldings.

PROCEDURES
Horses were randomly assigned (3/group) to first receive topical treatment of the left eye with 1% atropine or artificial tears solution; the right eye was left untreated. After 24 hours of treatment every 6 hours, 200 nontoxic beads were administered to each horse via nasogastric intubation and treatment frequency was decreased to every 12 hours for 4 more days. Pupillary light reflexes (PLRs), mydriasis, heart rate, fecal bead passage, abdominal girth measurements, auscultable gut sounds, fecal weight, and clinical signs of abdominal pain were monitored. Following a 4-week washout period, horses received the opposite treatment in the left eye and measurements were repeated. Serum atropine concentration (reflecting systemic absorption) was measured with an ELISA at various points after initial atropine administration.

RESULTS
No horse had subjective or objective evidence of colic or ileus at any monitoring point. Complete mydriasis of the left eye with absence of the PLR was identified in 5 horses within 6 hours and in all 6 horses within 12 hours after initial atropine administration. One horse had mydriasis with an absent PLR in the untreated eye by day 5 of atropine treatment. At no point was atropine detected in serum samples of any horse.

CONCLUSIONS AND CLINICAL RELEVANCE
Topical atropine application at clinically appropriate doses induced no evidence of ileus in healthy horses. (J Am Vet Med Assoc 2017;251:1324–1330)

Atropine sulfate antagonizes parasympathetic function by inhibiting the action of acetylcholine on autonomic receptors. Administration of the drug can cause various effects, including tachycardia and slowing of gut motility. Topical administration of atropine is an established treatment for horses with uveitis and corneal disease. Typically, the drug is used at a 1% concentration and is applied topically to the eye at a frequency up to every 4 to 6 hours. Once the pupil becomes maximally dilated, the administration frequency is decreased to that necessary to maintain pupillary dilation.

Topical atropine administration has 3 main functions in the treatment of intraocular inflammation. First, the drug provides analgesia by inhibiting spasms of the ciliary body muscle. Second, atropine causes mydriasis, decreasing the probability of synechia formation. Synechia can obstruct aqueous humor outflow, resulting in secondary glaucoma. Finally, atropine may help to stabilize the blood-aqueous barrier, thereby decreasing the leakage of inflammatory cells and protein into the anterior chamber. This infiltrate may also obstruct the normal outflow of aqueous humor and contribute to secondary glaucoma. Additionally, the presence of inflammatory cells in the eye worsens the clinical situation because these cells release free radicals and cytokines. Prompt and aggressive treatment of uveitis with atropine is therefore of paramount importance to minimize the risk of long-term sequelae.

Atropine use in horses has been associated with prolongation of gastrointestinal transit time, particularly within the colon and small intestine. Following IV atropine administration, decreases occur in the frequency of borborygmi and electrical activity of the cecum and ileum. Atropine-induced cecal impactions secondary to topical ophthalmic administration have also been reported. Although the
benefits of topical atropine administration are numerous, limited information exists regarding the effect of topically applied atropine on the intestines of horses in situ. Whereas several studies have been reported in which the effects of atropine administration to horses were subjectively evaluated, to date only 1 preliminary study (an abstract) has been reported in which the degree of gastrointestinal ileus associated with topical ophthalmic atropine administration was evaluated. Additionally, the percentage of topically applied atropine that is absorbed systemically when ocular barriers are present is unknown.

The purpose of the study reported here was to determine whether topical ophthalmic atropine administration would cause ileus in horses, as evidenced by clinical signs or slowing of gastrointestinal transit of small plastic beads, and whether such administration would result in quantifiable amounts of atropine in systemic circulation. Whereas some degree of ileus could be expected owing to factors that may predispose a horse to colic (eg, transportation or environmental or feed changes), we hypothesized that topical ophthalmic atropine administration would neither result in substantial serum atropine concentrations nor cause a significant decrease in gastrointestinal motility in healthy horses.

Materials and Methods

Animals

Six horses were obtained from the teaching and research herd of The Ohio State University College of Veterinary Medicine. Horses were geldings of various types (3 Warmblood crosses, 2 Quarter Horse crosses, and 1 mixed breed), with a mean age of 12.8 years (range, 7 to 25 years) and mean body weight of 425.4 kg (997 lb; range, 345 to 568 kg [759 to 1,250 lb]). All horses had darkly pigmented irides. All procedures were performed in accordance with the Association for Research in Vision and Ophthalmology Statement for the Use of Animals in Ophthalmic and Vision Research, and the study protocol was approved by The Ohio State University Institutional Animal Care and Use Committee.

Experimental design

A randomized, controlled, masked crossover design was used for the study and was intended to take into consideration the variable effects of atropine administration in horses. Because maximum pupil dilation may not be achieved in healthy horses for up to 2 days after topical atropine administration, an ophthalmic treatment period of 24 hours was chosen to be provided before beginning any objective gut motility measurements. Given that the effects of ophthalmically administered atropine can persist in horses for ≥ 14 days, a 4-week washout period was deemed necessary to ensure that atropine had been systemically cleared between treatment phases.

The 6 horses were randomly assigned identification labels of A through F by drawing letters from a hat. Horses were transported from pasture, allowed to acclimate to their individual stalls for 24 hours, and given water ad libitum and timothy hay twice daily, fed on the basis of nutritional maintenance guidelines for forage of 1.5% body weight/d. Initially, horses were housed on straw. However, before commencement of treatment phase 1 and for the duration of the study, horses were housed on stall mats with no bedding.

Following the acclimation period, the baseline transit time for bead passage through the gastrointestinal tract was determined for each horse to serve as an intrahorse comparison for subsequent measurements (baseline phase). For this procedure, horses were sedated with xylazine hydrochloride (0.4 to 0.5 mg/kg [0.18 to 0.23 mg/lb]) administered IV and 200 nontoxic plastic 3-mm beads were administered via nasogastric tube. These beads were shown to be safe in previous studies, with complete bead passage reportedly achieved within 3 days after administration. Horses were kept in their stalls following bead administration, fed hay twice daily, and given free access to water. Physical examinations were performed twice daily; feces were collected and weighed, and beads were retrieved and counted every 24 hours for 120 hours. Because atropine sensitivity varies among horses, this baseline measurement was intended to establish the typical amount of time required for ingesta passage in individual horses.

Following completion of baseline measurements, treatment phase 1 began, in which horses A, C, and E (group A) were randomly assigned to topical treatment with 0.1 mL (1 mg) of 1% atropine ophthalmic solution and horses B, D, and F (group B) to topical treatment with 0.1 mL of artificial tear solution. All treatments were applied to the left eye every 6 hours for the first 24 hours, and then the administration frequency was decreased to every 12 hours for the next 96 hours because pupillary dilation was expected within 24 hours after treatment was initiated. The right eye was left untreated. Topical administration of the drug was performed via a 1-mL tuberculin syringe with the needle removed by the same investigators, who were masked to treatment identity.

Following treatment phase 1, horses were turned out to pasture for the 4-week washout period, after which treatment phase 2 commenced. Horses were again allowed to acclimate to their individual stalls for 24 hours. Afterward, the same procedures used in phase 1 were used in phase 2, with the exception that horses in group A received the artificial tear treatment and those in group B received the atropine treatment as described for phase 1. At the completion of phase 2, horses were returned to their herd.

Monitoring and sample collection

Jugular venous blood samples were collected via 22-gauge needle into 12-mL syringes. Samples were then immediately transferred into red-top tubes and allowed to clot for 3 hours at room temperature (approx 22°C). This sample collection was performed immedi-
ately before treatment administration (0 hours); 6, 12, and 24 hours after administration; and then every 24 hours after treatment administration for the duration of the treatment phase. Samples were centrifuged at 3,000 × g at 4°C to obtain serum, and serum aliquots were stored at −20°C pending analysis.

Horses were monitored every 2 hours throughout each treatment phase for clinical signs of abdominal pain (eg, flank watching, rolling, pawing, kicking, or apparent lack of appetite) and a decrease in fecal output that would indicate a decrease in gut motility. Pupillary light reflexes and presence or absence of mydriasis were monitored by masked investigators every 6 hours for the first 24 hours, and then every 12 hours for the remainder of the treatment phase.

A masked investigator also monitored all horses every 12 hours in both treatment phases for heart rate and rhythm (via auscultation), gut motility (via auscultation), abdominal girth, and vertical pupil diameter. For auscultation of gut motility, 4 abdominal quadrants were auscultated (right and left ventral aspect of the abdomen and right and left paralumbar fossa). Each quadrant was graded 0 through 2 on the basis of the frequency with which borborygmi were detected (0 = no gut sounds, 1 = 1 sound every 5 seconds [normal gut motility], and 2 = ≥ 2 sounds every 5 seconds [increased gut motility]).

For measurements of abdominal girth, an equine body weight tape was used to measure the horse’s flank region to determine whether abdominal distention was present. Consistency was maintained by marking the paralumbar fossa bilaterally with a permanent marker to ensure the same region was measured each time.

Measurements of vertical pupil diameter by use of a Schirmer tear test strip were conducted every 12 hours by 1 investigator (RFW), who was also masked to treatment assignment. Additionally, photos were obtained in reference to a scale bar (Schirmer tear test strip) at 0, 12, 36, and 168 hours after treatment administration, and a masked grader (HLC) quantified vertical pupil diameter with the aid of imaging software.

Twenty-four hours after treatment was initiated in each treatment phase, horses were again sedated as for bead administration in the baseline phase and 200 nontoxic plastic 3-mm beads of a color different from that administered during the baseline and other treatment phases were administered to each horse via nasogastric tube. Afterward, fecal piles were counted and weighed, and beads were collected every 24 hours for 120 hours. Both phases were considered complete once bead passage ceased, which was by the 120-hour follow-up period for all horses.

**Serum atropine measurement**

Atropine concentration in serum samples was measured at the conclusion of sample collection for each treatment phase by use of an ELISA previously validated for use with equine serum. The maximum and minimum detection limits for equine serum were 5 ng/mL and < 1.0 ng/mL, respectively.

**Statistical analysis**

Data were summarized and differences in vertical pupil diameter, gastrointestinal transit times, fecal weight, and bead retrieval were analyzed with statistical software. To compare normally distributed continuous data between and within treatments, 2-way repeated-measures ANOVA and ANOVA with Bonferroni post hoc analysis were used. The Pearson χ² test was used to compare distributions of categorical data. Values of *P* ≤ 0.05 were considered significant.

**Results**

**Animals**

All 6 geldings completed all 3 phases of the study. No signs of colic (including changes in water or hay intake) were detected in any horse at any point. Gut motility remained consistent for each horse throughout the study. Every horse received a score of 1 (ie, normal) for all quadrants for all measurements. No significant change in flank measurements was identified in any horse at any assessment point. Heart rate of each horse remained consistent and no tachycardia (heart rate > 60 beats/min) was evident at any assessment point (mean ± SD heart rate, 38 ± 4 beats/min). No changes in heart rhythm were auscultated.

**Comparisons between treatments**

No significant differences between topical atropine and placebo treatments were identified for any monitored variable except pupillary dilation, nor was any difference identified in gastrointestinal transit times measured via bead passage between the baseline phase and atropine treatment or between atropine and placebo treatments. Complete mydriasis and absence of the PLR in the left (treated) eye was detected in 5 horses within 6 hours and in 6 horses within 12 hours after initiation of atropine treatment. One horse (horse F) had mydriasis (8-mm vertical pupil diameter) in the untreated right eye by 84 hours of atropine treatment. By 108 hours, this untreated eye had an 8.5-mm vertical pupil diameter and an absent PLR. The last dose of atropine was administered at 108 hours. By 120 hours, the untreated eye had an 8.5-mm vertical pupil diameter and a sluggish PLR.

In all horses, the study’s maximum vertical pupil diameter of 18-mm was achieved with continued atropine administration. An 18-mm vertical pupil diameter was achieved in 3 horses at 72 hours, 4 horses at 96 hours, 5 horses at 120 hours, and 6 horses at 132 hours after initiation of atropine treatment. Mean vertical pupil diameters in atropine-treated left eyes were significantly (*P* < 0.001) larger than in untreated control right eyes. At study completion (92 hours after atropine treatment was initiated and 72 hours after it concluded), atropine-treated eyes of 2 horses had a vertical pupil diameter of 15 mm and those of 4 horses had a vertical pupil diameter of 14 mm (Figure 1).
Feces production and gastrointestinal transit time

Mean ± SD values for mean daily fecal weight were 19.0 ± 2.8 kg (41.8 ± 6.2 lb) before treatment was initiated (baseline phase), 20.3 ± 1.7 kg (44.7 ± 3.7 lb) during atropine treatment, and 21.6 ± 4.6 kg (47.5 ± 10.1 lb) during placebo treatment. These values did not differ significantly (overall \( P = 0.41 \); baseline vs atropine, \( P = 0.36 \); baseline vs placebo, \( P = 0.27 \); baseline vs atropine, \( P = 0.52 \); Figure 2).

No significant differences were identified in mean number of beads retrieved from feces during the baseline phase and atropine and placebo treatments (overall \( P = 0.51 \); baseline vs atropine, \( P = 0.055 \); baseline vs placebo, \( P = 0.08 \); atropine vs placebo, \( P = 0.71 \)). Mean ± SD number of beads retrieved for all 6 horses was 138.5 ± 29.6 for the baseline phase, 170.7 ± 21.0 for atropine treatment, and 163.8 ± 12.2 for placebo treatment (Figure 3).

For all 3 phases, day 0 (day of bead administration) yielded no beads. The day following bead administration (day 1), beads first appeared in feces in all 3 phases. Mean ± SD bead counts were 11 ± 12.3 for the baseline phase, 22.0 ± 0 for atropine treatment, and 20.2 ± 26.5 for placebo treatment (overall \( P = 0.50 \); baseline vs atropine, \( P = 0.47 \); baseline vs placebo, \( P = 0.46 \); atropine vs placebo, \( P = 0.92 \)). Day 2 represented the day of maximum bead retrieval for all 3 comparison groups, with mean ± SD bead counts of 109.7 ± 32.5, 83.8 ± 42.3, and 111.8 ± 54.2 for baseline phase, atropine treatment, and placebo treatment, respectively (overall \( P = 0.49 \); baseline vs atropine, \( P = 0.26 \); baseline vs placebo, \( P = 0.94 \); atropine vs placebo, \( P = 0.34 \)). Mean ± SD bead counts for day 4 were 17.2 ± 12.3, 62.5 ± 53.9, and 19.7 ± 13.2 for baseline phase, atropine treatment, and placebo treatment, respectively (overall \( P = 0.055 \); baseline vs atropine, \( P = 0.07 \); baseline vs placebo, \( P = 0.74 \); atropine vs placebo, \( P = 0.09 \)). Mean ± SD bead counts for day 5 were 0.7 ± 1.0, 2.3 ± 2.4, and 1.0 ± 1.1, for baseline phase, atropine treatment, and placebo treatment, respectively (overall \( P = 0.20 \); baseline vs atropine, \( P = 0.14 \); baseline vs placebo, \( P = 0.60 \); atropine vs placebo, \( P = 0.24 \)). No beads were retrieved on day 5 after bead administration for during any phase.

Serum atropine concentration

No atropine was detected by the ELISA in any serum sample from any horse, regardless of the assessment point (0, 6, 12, 24, 48, 72, 96, 120, 142, and 164 hours after treatment initiation).
The clinically normal horses enrolled in the present study had negligible systemic uptake of atropine. Topically applied drugs eventually pass into the peripheral circulation by absorption at several sites: the conjunctiva and cornea comprising the ocular surface and then the nasolacrimal drainage system. Drugs, including atropine, can be readily absorbed into the blood vessels of the conjunctiva and then the nasolacrimal drainage system. Topically applied ophthalmic drugs will also traverse the nasolacrimal drainage system where, despite a decrease in surface area relative to the conjunctiva, drugs can have prolonged retention and can subsequently be absorbed through the nasal mucosa or gastrointestinal system. The nasolacrimal drainage system and nasal mucosa contribute considerably to the systemic absorption of atropine in humans and rabbits. In these 2 species, the lipophilic nature and small molecular weight of atropine is believed to facilitate drug absorption into the mucous membranes associated with tear-film kinetics.

In the present study, horses had intact corneal epithelium and ocular barriers; however, horses with keratitis or uveitis may have a disrupted corneal epithelial barrier or blood-aqueous barrier. The potential for disruption of the ocular barrier in diseased states should be taken into account when applying the study results to clinical scenarios, given that this situation may allow increased passage of topically applied drugs into systemic circulation.

In the present study, horses had mydriasis and loss of the PLR in the untreated right eye by 108 hours of atropine treatment of the left eye, which was observed in no other horse. This contralateral involvement indicated systemic absorption of atropine. Although the minimum detection limit of the ELISA used for measurement of atropine concentration in equine serum samples was 1.0 ng/mL, the serum concentration (and thus systemic concentration) of atropine in this horse was below this limit, raising the possibility that if atropine were present in systemic circulation, the serum concentration may have been outside the limits of detection. By comparison, in rabbits that receive topically applied atropine in a single 0.25-mg dose, peak plasma atropine concentrations (measured in picograms per milliliter) are appreciable 30 minutes after administration. In humans, topical administration of a single 0.4-mg dose of atropine results in peak plasma concentrations 8 minutes after administration.

To the authors’ knowledge, no pharmacokinetic studies have been reported in which systemic uptake of atropine was quantified following topical administration to horses.

In species other than horses, atropine is known to rapidly distribute throughout the body and move quickly out of systemic circulation. In humans, most atropine is excreted in the urine within the first 12 hours after IV administration, and 50% of the original dose is excreted within 24 hours. Although blood samples were collected at multiple points from

Discussion

Ocular disorders are among the most common health problems in horses, and immune-mediated recurrent uveitis is the leading cause of blindness. Repeated episodes of uveitis induce ocular pain and blinding sequelae, such as synchiae, cataracts, retinal detachment, and secondary glaucoma. The prevalence of recurrent uveitis in horses has been reported to be as high as 25% in the United States and 40% in parts of Europe. Owing to the ability of atropine to alleviate ocular pain and reduce intraocular inflammation, the drug is considered important in the treatment of common equine eye diseases, such as recurrent uveitis and ulcerative and infectious corneal disease.

The clinically normal horses enrolled in the present study had negligible systemic uptake of atropine. Topically applied drugs eventually pass into the peripheral circulation by absorption at several sites: the conjunctiva and cornea comprising the ocular surface and then the nasolacrimal drainage system. Drugs, including atropine, can be readily absorbed into the blood vessels of the conjunctiva and removed from the ocular surface into systemic circulation. If the intended target of a topically applied drug is located intraocularly, the drug must overcome the tight junctions of the corneal epithelium and the hydrophilic nature of the corneal stroma. Successful passage through the corneal barriers permits drug access into the aqueous humor, and from there, the drug can then enter systemic circulation through aqueous humor outflow and uveal blood vessels.
each horse in the present study for serum atropine analysis, systemic atropine concentrations may have peaked earlier than the first sample collection point. Alternatively, if peak systemic atropine concentration occurred late, the sample collection points may have been spread out too far to detect systemic absorption. Additional blood sample collections, particularly at earlier points, are warranted to fully evaluate the extent of systemic atropine absorption following topical ophthalmic administration.

In the study reported here, horses received 4 mg (four 1-mg doses) of atropine over a 24-hour period and a total dose of 12 mg of atropine over the 120-hour treatment duration. At a mean horse body weight of 450 kg (990 lb), this dose would have been equivalent to 0.009 mg/kg (0.004 mg/lb) over a 24-hour period or a total cumulative dose of 0.0267 mg/kg (0.012 mg/lb). Although these values are not historically compatible with signs of colic in horses, the present study involved healthy horses. Eyes with iridocyclitis are reportedly more resistant to atropinization than clinically normal eyes, potentially requiring higher atropine doses or more frequent administration to achieve the desired outcome. As such, further objective evaluation is warranted of the systemic effects of topically applied atropine on diseased eyes and the amount that is absorbed systemically.

Signs of colic were not observed in any of the horses during the present study. Intravenous injections of atropine have been associated with signs of colic and slowing of gastrointestinal motility in horses. Other studies have been conducted to evaluate whether colic is associated with topically applied atropine. In 1 study, 0.1 mL (1 mg) of atropine was unilaterally applied to 6 horses every hour for 24 hours. Four horses developed signs of colic, 2 of which required unspecified treatment. Atropine administration at this frequency is not typically used clinically. In a different study, atropine was topically applied unilaterally to 4 horses in a crossover study design. No horse developed signs of colic when 1 drop of 1% atropine solution was administered every 3 hours for 48 hours or when 1 drop was administered every 12 hours for the next 72 hours. Although this administration regimen is clinically relevant, 1 drop of atropine is equivalent to only 0.05 mL (0.5 mg) of the drug, which is considered a lower atropine dose. Neither of these 2 studies involved the use of objective measurements to quantify the degree of ileus; the investigators relied on subjective evaluation involving auscultation to grade gut sounds.

Two retrospective studies have been reported involving horses hospitalized for ocular disease and receiving topical atropine treatment. In 1 study, 15 horses received atropine topically at solution concentrations ranging from 0.25 to 1% and a frequency ranging from every 12 hours to continuous lavage. No horses developed signs of abdominal discomfort or had a decrease in intestinal motility. In the second study, topical atropine administration was not a risk factor for colic in 337 horses hospitalized for ocular disease. Instead, duration of hospitalization and age were associated with the risk of colic.

Because the present study was preliminary in nature, certain variables were controlled for to reduce the influence of possible confounders, including iris pigmentation (all had darkly pigmented irides) and sex and reproductive status (all were geldings). Studies have shown that darkly pigmented individuals have a longer duration of effects of topically applied drugs than other individuals; this has been associated with a greater binding affinity to the melanocyte. The atropine-bound melelin serves as a drug depot, gradually releasing the molecule to cause a prolonged mydriatic effect and potentially resulting in less free atropine in systemic circulation. Additionally, the effects of atropine can differ with age, breed, and sex. For example, mares appear to be more atropine responsive than male horses. In another study involving topical atropine administration, Arabians reportedly develop mydriasis for a longer duration than horses of other breeds; however, because only Arabian mares were used in the associated study, it remains unknown whether the effects would be similar for males.

The present study had several limitations. First, with a sample size of 6 clinically normal horses, observations and findings from this study cannot be extrapolated to the general horse population. Although a larger sample size might have increased the power to detect significant differences that actually existed but were missed, the authors feel confident that the described topical use of atropine resulted in no signs of colic. Our objective findings supported most previously reported subjective findings for the same hypothesis. Second, bead retrieval from feces was challenging. The bead loss that occurred during the baseline phase was likely due to the horses being housed on straw, making bead collection much more difficult. Although the difference was not significant, fewer total beads were retrieved from each horse during the baseline phase than during the treatment phases. This variable was addressed prior to commencement of phase 1, during which horses were housed on stall mats only.

The objectively measured outcomes in the study reported here suggested that topical atropine administration to the eyes of clinically normal horses resulted in no detectable systemic concentration of the drug and caused no ileus or clinical signs of colic. As such, atropine treatment as described here when indicated in horses with ophthalmic diseases such as uveitis or corneal disease should have a low probability of inducing colic. However, such conclusions can only be definitively made following evaluation of the systemic effects of topically applied atropine in horses with ophthalmic disease. Once mydriasis and cycloplegia are present, the frequency of topical atropine administration should be reduced to maintain pupillary dilation but ideally avoid systemic effects or mydriasis in the untreated eye. With a clinically
relevant administration regimen, the adverse effects associated with topical ophthalmic atropine administration should be minimal in systemically healthy horses.

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Footnotes

5. Image J software, National Institutes of Health, Bethesda, Md.
6. IDEL-F008 forensic atropine kit, Empire Genomics LLC, Buffalo, NY.
7. GraphPad for Windows Software, La Jolla, Calif.

References