Pharmacokinetics and pharmacodynamics of olopatadine following administration via nasogastric tube to healthy horses

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OBJECTIVE
To investigate the pharmacokinetics and antihistaminic effects (pharmacodynamics) of olopatadine in a small population of healthy horses after administration via nasogastric tube.

ANIMALS
4 healthy adult Thoroughbreds.

PROCEDURES
Olopatadine (0.1 mg/kg, once) was administered via nasogastric tube. Blood samples were collected at predetermined time points for pharmacokinetic analyses of the drug in plasma. Olopatadine effects were investigated by measurement of cutaneous wheals induced by ID histamine injection (0.1 mL [10 μg]/injection) at predetermined time points. Inhibition effect ratios were calculated on the basis of measured wheal size (area) after versus before olopatadine administration.

RESULTS
Mean ± SD maximum plasma olopatadine concentration was 48.8 ± 11.0 ng/mL approximately 1.5 hours after administration. Median terminal half-life was 6.11 hours. Mean ± SD maximal effect was 88.2 ± 4.9% inhibition approximately 3.5 hours after drug delivery, and the inhibition effect remained > 80% for 12.5 hours after treatment. No signs of adverse clinical effects were observed.

CONCLUSIONS AND CLINICAL RELEVANCE
Results suggested olopatadine may have a strong, long-term inhibitory effect against histamine-induced wheals in the skin of horses. Clinical research with a larger number of horses is warranted. (*Am J Vet Res* 2019;80:689–695)

Histamine 1 receptor antagonists (antihistamines) are currently used as treatments for urticaria and various other allergy symptoms in human and veterinary medicine.[1-5] However, first-generation antihistamines can cause adverse CNS effects, including depression.[6] Some more recently developed second-generation antihistamines do not readily cross the blood-brain barrier and have fewer adverse effects, and these are commonly used to treat human patients.[7-9]

Olopatadine hydrochloride is a second-generation antihistamine that is widely administered to people orally in tablet form.[3,10–13] Olopatadine inhibits the release of inflammatory lipid mediators from polymorphonuclear leukocytes and eosinophils[11] and occupies fewer H_1 receptors in the brain than other antihistamines.[14] In a double-blind randomized controlled study,[15] orally administered olopatadine produced more potent and stable antihistaminic effects on skin responses than did other second-generation antihistamines at standard doses in human patients.

Horses are known to develop allergic disorders that are similar to those in people,[16,17] and inexpensive first-generation antihistamines have been predominantly used for medical treatment in this species.[5,18,19] Two first-generation antihistamines, D-chlorpheniramine and clemastine, were found to exert insufficient antihistaminic effects in the skin of horses with histamine-induced cutaneous wheal formation at the tested dosages.[18,19] In recent years, the pharmacokinetic and pharmacodynamic properties of second-generation antihistamines including fexofenadine and cetirizine[20–22] have been evaluated in horses, and efficacy in the treatment of allergic disorders has been reported. However, to the authors' knowledge, the pharmacokinetics and pharmacodynamics of olopatadine in horses have not been investigated. The purpose of the study reported here was to estimate the pharmacokinetic parameters of olopa-

ABBREVIATIONS

C_{max}	Maximum observed concentration
H_1	Histamine 1
HPLC	High-performance liquid chromatography
LC-MS	Liquid chromatography-mass spectrometry
t_{max}	Time to maximum concentration

tadine and to evaluate its antihistaminic effects after administration via nasogastric tube to a small number of healthy horses with histamine-induced cutaneous wheal formation.

Materials and Methods

Animals

Four healthy Thoroughbreds (2 stallions and 2 mares; median age, 3.5 years [range, 3 to 5 years]; median body weight, 442.5 kg [range, 400 to 478 kg]) were used in the study. The horses belonged to the Japan Racing Association and were kept indoors in individual stalls during the experiments with ad libitum access to timothy hay and water. All experiments were approved by the Animal Care and Use Committee of the Miho Training Center Equine Clinic, Japan Racing Association (09 502 K2 01 00014).

Experimental protocol

Injections, reaction site examinations and measurements, and blood sample collection were performed by 2 investigators (TK and NT). Tests were performed according to previously described protocols.[18]

Immediately prior to the first experiment, the skin on both sides of the neck (from the base to the midcervical region; approx 30 × 40 cm) was clipped with electric clippers and washed with iodine scrub and water. Histamine hydrochloride[a] was dissolved in sterile saline (0.9% NaCl) solution to a final concentration of 100 µg/mL for ID administration. This solution was prepared on the first day of testing, kept protected from light with refrigeration (4°C), and used within 4 days. All injections were performed with a 27-gauge needle and 1-mL syringe. Hair over the left jugular vein was clipped with electric clippers, and following preparation as previously described, a 14-gauge IV catheter[b] was placed in the left jugular vein for blood sample collection.

In a pilot investigation, cutaneous wheals resulting from ID histamine administration in horses reached maximum size approximately 20 minutes after injection and disappeared within approximately 1 hour after injection (unpublished data). Therefore, we measured each wheal once, 20 minutes after ID injection, in the present study. All measurements were performed with digital calipers.[c] One measurement was made of each wheal across its greatest diameter, and a second measurement was made along a line perpendicular to the direction of the first measurement. Wheal sizes were calculated from the measured diameters by use of the formula for the area of an ellipse.

Control experiment—Thirty minutes prior to olopatadine administration, a control experiment was conducted. Each horse received 2 sets of ID injections performed simultaneously; 0.1 mL (10 µg) of histamine solution was administered on the left and right sides of the neck (3 replicates/side), and 0.1 mL of sterile saline solution was administered on the left and right sides of the neck (3 replicates/side). Diameters of the wheals of each type were measured as described in 6 replicates for each horse. The mean measured area of the wheal induced by ID injection of histamine in the absence of olopatadine was used as a measure of 0% inhibition, representing the local reaction with no blockage of H_1 receptors by antihistamine. The mean area of the wheal resulting from saline treatment was used as a measure of 100% inhibition, representing the effects of local injection with all H_1 receptors blocked by antihistamine. The percentage inhibition of wheals that developed in response to histamine administration after olopatadine treatment in the subsequent experiments was calculated by comparison with the areas representing 0% and 100% inhibition. The posttreatment measurements were compared with the mean area of wheals in the control study for each horse by use of the following formula:

Inhibition rate (%) = (mean area at 0% inhibition – mean area of the wheal following ID histamine injection at time *t*)/(mean area at 0% inhibition – mean area at 100% inhibition)•100.

Administration of olopatadine—Olopatadine was administered to each horse once at a dose of 0.1 mg/kg, approximating doses that are commonly used in human studies of efficacy and safety.[10,23] Olopatadine tablets[d] were crushed, weighed with an electric scale to achieve the desired dose, suspended in 500 mL of water, and administered via nasogastric tube. The tube was flushed with 500 mL of water immediately after drug administration.

Blood sample collection—Blood samples were collected through the jugular catheter at time 0 (immediately prior to olopatadine administration) and 0.1, 0.2, 0.3, 0.5, 1, 1.5, 2, 4, 6, 9, 12.5, 23, 30, and 36 hours after olopatadine administration. The samples were collected into heparin-containing vacuum tubes.[e] Samples were immediately centrifuged at 1,500 × *g* for 10 minutes, and separated plasma samples were stored at −20°C until analysis. The olopatadine assay was performed ≤ 1 month after blood sample collection.

Skin testing after olopatadine administration—The antihistaminic effects of olopatadine were estimated by evaluation of wheal sizes following ID injections of histamine. Histamine injections (0.1 mL [10 µg]) were performed as described in the prepared lateral regions of the neck. Duplicate injections were performed simultaneously on the right and left sides of the neck 0.5, 1, 2, 4, 6, 12.5, 23, 36, 48, and 72 hours after olopatadine treatment, and each wheal diameter was measured as described 20 minutes after each injection. Wheal sites were indicated with a marker, and a 50-mm distance was left between sites to prevent overlap.

Table 1—Summary of intraday and interday measures of precision (coefficient of variation) and accuracy (relative error) for detection of olopatadine in equine plasma.

Measurement	Concentration (ng/mL)	Mean ± SD calculated concentration (ng/mL)	Coefficient of variation (%)	Relative error (%)
Intraday	0.4	0.44 ± 0.04	9.14	10.7
	1	0.98 ± 0.07	6.66	−2.10
	10	9.28 ± 0.19	2.09	−7.18
	100	101 ± 4.14	4.10	0.88
Interday				
	0.4	0.41 ± 0.07	15.9	2.42
	1	1.01 ± 0.07	6.82	1.19
	10	9.80 ± 1.01	10.31	−2.03
	100	100 ± 0.86	0.86	0.43

Clinical monitoring—All horses were evaluated for signs of adverse effects 0.5, 1, 2, 4, 6, 12.5, 23, 36, 48, and 72 hours after olopatadine administration. Sedation was evaluated by 2 veterinarians (TK and NT) on the basis of position of the head and various behaviors, including responses to abrupt noise (produced by clanging metal rings), blood sample collection, and injection of histamine. Sedation was recorded as none, mild, moderate, or severe. In addition, gastrointestinal function was monitored by observation and recording of the appetite of each horse and properties of feces.

Olopatadine assay

Olopatadine concentrations in plasma samples were measured by means of LC-MS with a mass spectrometer[f] equipped with an HPLC system.[g] A 30-µm extraction cartridge[h] (60 mg of sorbent; 3 mL) was conditioned with 3 mL of pure methanol, followed by 3 mL of water, before sample loading. Then, 0.1-mL aliquots of cyproheptadine hydrochloride[i] (100 ng/mL in water) were added to 1-mL stored plasma samples as an internal standard, and each sample was loaded into a conditioned extraction cartridge. The cartridge was washed with 3 mL of 20% methanol and eluted with 3 mL of pure methanol; the eluates were evaporated to dryness at 40°C under a stream of nitrogen gas. The resulting residues were reconstituted in 200-µL aliquots of 50% methanol, and 20-µL aliquots were used in LC-MS analysis.

Separation of extracted analytes was achieved with a C18 column[j] (150 mm × 2.1 mm; internal diameter, 5 µm). The mobile phase comprised a blend of 0.1% formic acid and methanol (vol/vol, 55:45) and was applied at a flow rate of 0.5 mL/min. The entire elutes were directed to the mass spectrometer, and analyses were performed with electrospray ionization in the positive ion mode. The selected-ion monitoring method was used for analysis of olopatadine (m/z ratio, 338) and cyproheptadine (m/z ratio, 288).

Olopatadine powder (purified up to 99.5%) was prepared for use as a reference standard by extracting the compound from olopatadine tablets.[d] Briefly, crushed tablets were dissolved in pure methanol, vortexed vigorously, and centrifuged at 1,940 × *g* for 5 minutes, and the methanol phases were collected and dried. The purity of extracted olopatadine was evaluated by means of HPLC[g] with UV detection at a wavelength of 254 nm and gas chromatography–mass spectrometry[k] (used in full scan mode). The olopatadine calibration curve and quality controls were prepared by spiking blank equine plasma with the reference standard. Calibration curves were plotted with olopatadine concentrations (x) versus the ratio of analyte peak area to internal standard peak area (y) and weighted (with a weighting factor of 1/y), and linear regression analyses[l] of the ratio of olopatadine peak areas and internal standard peak areas were performed. The final calibration curve had a coefficient of correlation (R^2) > 0.995 over the concen-

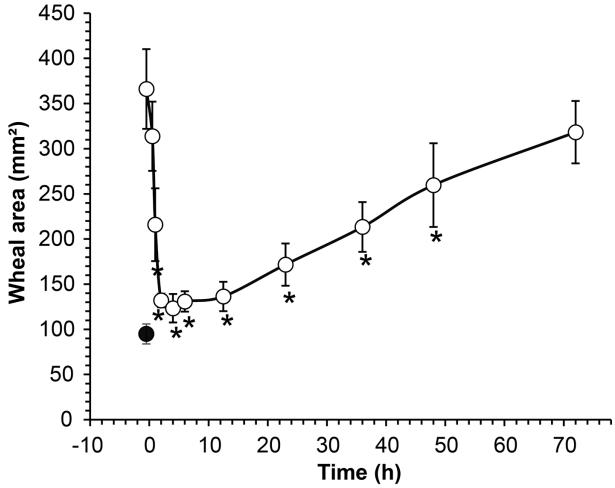

Figure 1—Mean ± SD area of cutaneous wheals induced by ID injection of histamine (0.1 mL [10 µg]/site/time point) in 4 healthy adult Thoroughbreds before (0% inhibition control; injection performed approx 30 minutes prior to drug delivery) and at predetermined time points after administration of olopatadine (0.1 mg/kg, once, via nasogastric tube) at time 0 (white circles). Histamine was administered ID simultaneously to both sides of the neck 0.5, 1, 2, 4, 6, 12.5, 23, 36, 48, and 72 hours after olopatadine administration, and measurements were performed 20 minutes after each injection. The mean of bilateral measurements for each horse at each time point were analyzed by 1-way repeated-measures ANOVA followed by a Dunnett post hoc test. Significant ($P < 0.01$) differences, compared with measurements at 0% inhibition, are indicated (asterisks). The mean ± SD area of cutaneous wheals induced by ID injection of 0.1 mL of sterile saline (0.9% NaCl) solution in 4 healthy adult Thoroughbreds before olopatadine administration (black circle) is also shown (100% inhibition control; injection performed approx 30 minutes prior to drug delivery).

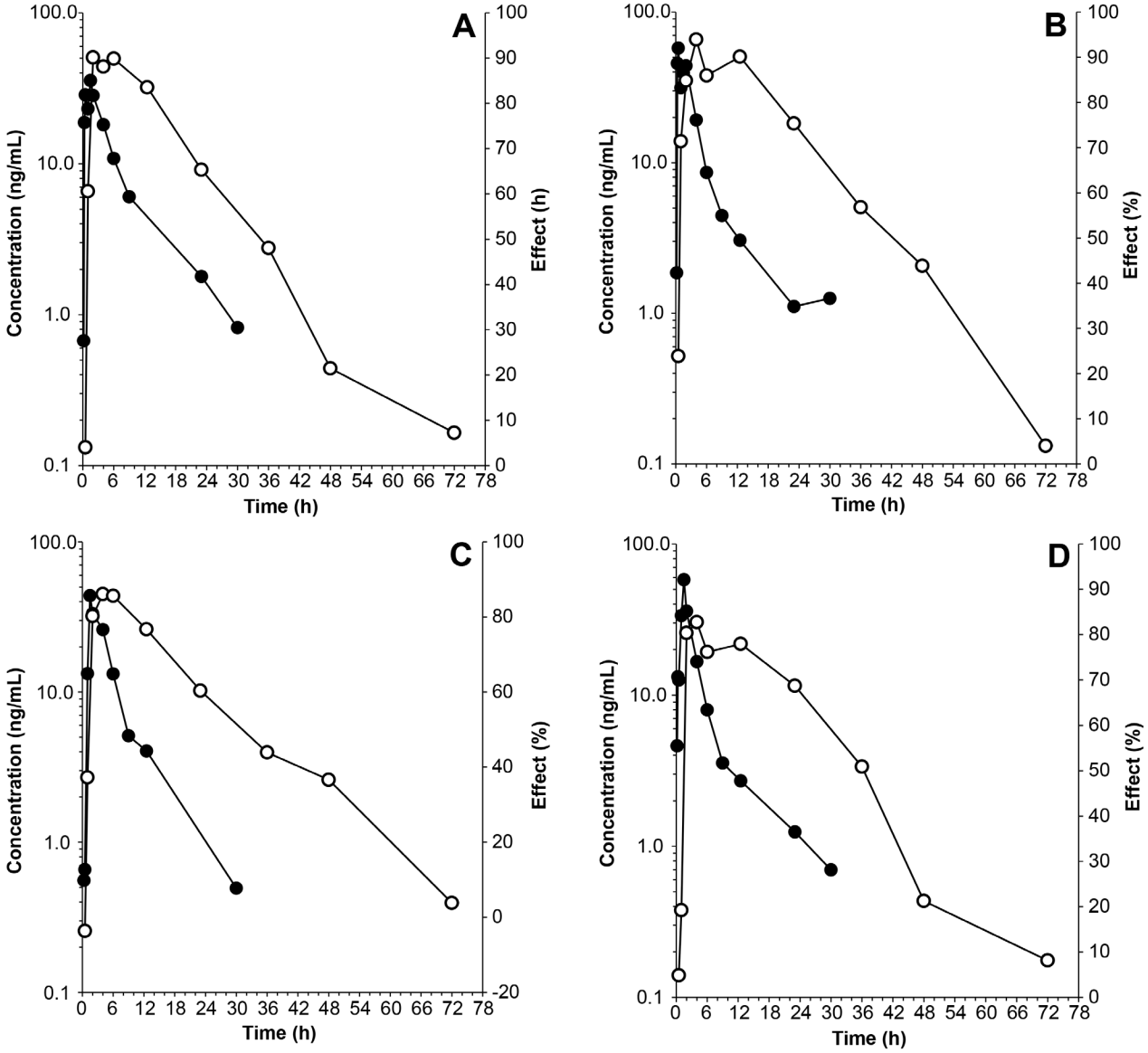

Figure 2—Plasma concentrations of olopatadine (black circles) and inhibitory effects of the drug on the measured area of histamine-induced wheals (white circles) for the same 4 horses as in Figure 1 (A through D). Each panel depicts results for 1 horse. Blood samples were collected immediately prior to (time 0) and 0.1, 0.2, 0.3, 0.5, 1, 1.5, 2, 4, 6, 9, 12.5, 23, 30, and 36 hours after olopatadine administration; the drug was undetectable at the last sampling point. Percentage inhibition of the histamine effect was calculated as (mean area at 0% inhibition – mean area of the wheal following ID histamine injection at time *t*)/(mean area at 0% inhibition – mean area at 100% inhibition)•100.

tration range of 0.4 to 100.0 ng/mL, and the lower limit of quantitation was 0.4 ng/mL. The recovery ratios for olopatadine in quality control samples were determined at concentrations of 1, 10, and 100 ng/mL (5 replicates each). Interday and intraday precision and accuracy were assessed with olopatadine quality control samples at concentrations of 0.4, 1, 10, and 100 ng/mL (5 replicates each). Intraday comparisons were performed over a period of 3 days. All standards and test samples were prepared in duplicate.

Pharmacokinetic analysis

The pharmacokinetic parameters of olopatadine in plasma after administration via nasogastric tube were estimated by noncompartmental analyses[m]; C_{max} and t_{max} were determined from plotted curves. The elimination rate constant was determined through use of log-linear regression of the final 4 data points in the terminal slope, and half-life values were calculated as ln2 divided by the elimination rate constant. The area under the plasma concentration-versus-time curve and the area under the first moment curve from time 0 to infinity were calculated with the trapezoidal method. The mean residence time was calculated as the area under the first moment curve divided by the area under the plasma concentration-versus-time curve.

Statistical analysis

Statistical analysis was conducted with commercially available software.[n] Olopatadine concentration

data and all pharmacodynamic data were normally distributed. Mean ± SD were calculated for histamine inhibition rate (%) and normally distributed pharmacokinetic parameters. Two pharmacokinetic parameters that were not normally distributed (half-life and t_{max}) were calculated as median and range. Comparisons of wheal areas before olopatadine treatment (controls) and after each posttreatment ID histamine injection were performed by 1-way repeated-measures ANOVA followed by a Dunnett post hoc test. Because right and left wheal areas did not differ significantly (Student *t* test, $P = 0.97$), the mean value for right and left wheal areas was taken for each horse at each time point. For all comparisons, values of $P < 0.01$ were considered significant.

Results

The mean ± SD recovery ratios for olopatadine quality control samples at concentrations of 1, 10, and 100 ng/mL were 95.6 ± 7.05%, 87.9 ± 5.30%, and 92.5 ± 9.57%, respectively. The intraday and interday precision and accuracy for detection of olopatadine in quality control samples were summarized **(Table 1)**.

Olopatadine appeared to be well tolerated by all study horses. No overt adverse drug effects such as signs of sedation or gastrointestinal dysfunction were observed in any of the horses.

The mean ± SD areas of cutaneous wheals induced by histamine before and up to 72 hours after administration of olopatadine (with the time of drug administration considered time 0) are shown **(Figure 1)**. The effect-versus-time curve revealed that mean wheal size was significantly smaller than that measured in the 0% inhibition control experiment from 1 to 48 hours after drug administration ($P < 0.001$ at 1 to 36 hours and $P = 0.001$ at 48 hours). The percentage inhibitions of wheals in each horse after olopatadine administration are shown **(Figure 2)**. The mean ± SD effects were maximal (88.2 ± 4.9% inhibition) at 3.5 ± 1.0 hours and were > 80% until 12.5 hours after drug administration. The concentration-versus-time curves revealed that olopatadine was detected in plasma from 0.3 to 30 hours after administration in all horses. The pharmacokinetic parameter estimates for olopatadine were summarized **(Table 2)**. A hysteresis plot of the relationship between plasma olopatadine concentrations and percentage inhibition of histamine-induced wheals revealed a counterclockwise hysteresis pattern **(Figure 3)**.

Table 2—Pharmacokinetic parameter estimates for olopatadine in plasma after administration via nasogastric tube (0.1 mg/kg, once) to 4 healthy adult Thoroughbreds in a study to investigate the pharmacokinetics and antihistaminic effects (pharmacodynamics) of the drug in horses.

Parameter	Result
AUC_{0-last} (ng•h/mL)	210 ± 15.0
$AUC_{0-\infty}$ (ng•h/mL)	220 ± 14.9
$AUMC_{0-last}$ (ng•h^2/mL)	1,194 ± 111
$AUMC_{0-\infty}$ (ng•h^2/mL)	1,566 ± 103
MRT (h)	7.14 ± 0.39
$t_{1/2}$ (h)	6.11 (6.07–9.01)
t_{max} (h)	1.5 (0.5–1.5)
C_{max} (ng/mL)	48.8 ± 11.0

Results are presented as mean ± SD or median (range).

$AUC_{0-\infty}$ = Area under the concentration-versus-time curve from time 0 to infinity. AUC_{0-last} = Area under the concentration-versus-time curve from time 0 to the last measured concentration. $AUMC_{0-\infty}$ = Area under the first moment curve from time 0 to infinity. $AUMC_{0-last}$ = Area under the first moment curve from time 0 to the last to the last measured concentration. MRT = Mean residence time. $t_{1/2}$ = Half-life.

Discussion

Following administration of olopatadine (0.1 mg/kg, via nasogastric tube) to a small number of healthy horses in the present study, antihistaminic effects were sustained for approximately 12 hours, and no obvious adverse effects were observed in any of the horses. In people, 5 mg of olopatadine can be administered orally every 12 hours with lesser sedation effects than reported for other antihistamines.[14,24] Similarly, the olopatadine doses administered in the present study were not associated with apparent signs of sedation.

The pharmacokinetic parameters of olopatadine after administration to people have been described.[11,23,25] In the present study, the median t_{max} of olopatadine in plasma was slightly later and mean C_{max} was slightly lower than has been reported for human plasma following treatment with similar doses.[11,23,25] These human studies were performed under

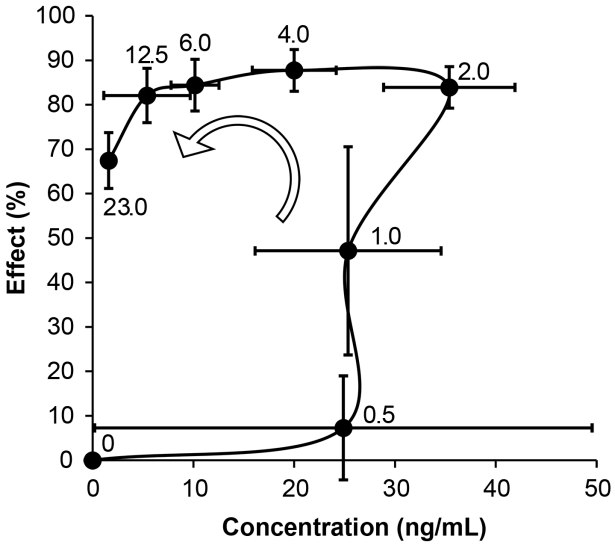

Figure 3—Hysteresis plot depicting the relationship between mean ± SD plasma concentrations of olopatadine and mean ± SD percentage inhibition of histamine-induced wheals for the 4 horses in Figure 1. The time (hours after olopatadine administration) at which blood samples were collected for plasma drug determination and wheal measurements were performed is indicated next to each data point. The arrow shows the direction of the passing of time. The data point at time 0 with 0% inhibition represents the plasma concentration of olopatadine and the 0% inhibition value determined just prior to nasogastric intubation and drug delivery.

fasting conditions, whereas our study horses were allowed free access to hay. Food consumption reportedly delayed the t_{max} and decreased the C_{max} of olopatadine following oral administration in people.[25] Thus, feeding may have influenced drug absorption in the horses of the present study. Because there could be differences between oral administration of tablets in the human study and administration of crushed tablets via nasogastric tube in the present study, these differences might also have affected the parameters.

Intradermal histamine tests have been routinely used to evaluate the effects of H_1 receptor antagonists in skin.[26,27] In people, these tests revealed > 90% inhibition of histamine-induced wheals for up to 8 hours after oral administration of olopatadine at 5 mg/person, and the antihistaminic effects of olopatadine were significantly greater than those of other antihistamines.[24,28] In the present study, mean inhibition of histamine-induced cutaneous wheal formation in horses was maximal (88.2%) 3.5 hours after olopatadine administration via nasogastric tube, and > 80% mean inhibition was observed for 12.5 hours. These results suggested that the antihistaminic effects of olopatadine in skin are approximately equivalent in horses and people. In humans, results following oral administration of olopatadine (5 mg, PO, q 12 h) indicated a high degree of clinical efficacy against chronic urticaria.[29] Administration of the drug via nasogastric tube at 0.1 mg/kg every 12 hours may be similarly effective against urticaria in horses. However, as the present study did not investigate repeated dosing, further research is necessary to assess safety during repeated administration.

In horses of a previous study,[19] clemastine at a dosage of 0.05 mg/kg, IV, resulted in a mean maximum wheal inhibition of 65%, but at a dosage of 0.2 mg/kg via nasogastric tube, substantially less inhibition (30%) was observed. Similarly in another study,[20] fexofenadine administered to horses at 0.7 mg/kg, IV, resulted in a mean maximum wheal inhibition of 70%, but at a dosage of 10 mg/kg via nasogastric tube, this value was considerably lower (55%). These reports confirmed that decreased effects after administration at high doses by this route resulted from low bioavailability (< 5.0%), and thus, oral administration of these drugs is not recommended in this species. For D-chlorpheniramine, observed mean maximal wheal inhibition at dosages of 0.5 mg/kg, IV and 0.5 mg/kg via nasogastric tube was 61% and 39%, respectively, with a bioavailability of 38% after the latter treatment.[18] However, the effect of 0.5 mg/kg via nasogastric tube administration was rapidly decreased to 15% inhibition 6 hours after treatment, and the authors concluded that the decrease resulted from rapid elimination and thus recommended frequent administration. Conversely, repeated cetirizine treatment (0.2 mg/kg, q 12 h via nasogastric tube) of horses resulted in mean minimum trough concentrations of 16 ng/mL and mean maximum wheal inhibition of 84%, maintained at > 50% for 11 hours after administration.[21] Antihistaminic effects may be influenced by various factors, such as drug concentrations in plasma or at the site of action and binding ability or binding duration at H_1 receptors, and it is difficult to conclude that differences in effects depend completely on pharmacokinetics. However, on the basis of our study results, olopatadine may have a strong and long-term histamine inhibition effect in the skin of horses. Clinical studies with sufficiently large sample sizes are warranted to evaluate the antihistaminic effect of olopatadine in equine patients.

Pharmacokinetic-pharmacodynamic link models can be used to measure drug potential through analyses of the relationship between drug concentrations and efficacies.[30,31] In the present study, the relationship between olopatadine concentrations and subsequent therapeutic effects followed a counterclockwise hysteresis loop with the passing of time after drug administration via nasogastric tube, with delayed effects relative to increases in plasma drug concentrations and maintenance of effects following decreases in plasma drug concentrations. Other antihistamines in horses had these results after administration by this route.[18–20] This may be a desirable property for antihistamines and may prolong the antihistaminic effect in horses. A counterclockwise hysteresis effect could result from a variety of underlying mechanisms such as delayed distribution to the site of effect or sustained drug presence at the site of effect, slow binding to or dissociation from the relevant receptors, and delayed pharmacological activity.[31] However, reports involving antihistamines are currently limited in this regard. Although a counterclockwise hysteresis loop has been reported for the effect of fexofenadine on the heart (assessed as QTc interval prolongation) versus the circulating drug concentration after administration to human subjects,[32] the mechanism involved in this process has not been elucidated. In a systematic review[33] of studies on the effects of fexofenadine, loratadine, and D-chlorpheniramine in people, distribution delay to the skin and sustained presence of the drug in skin were reported. Although concentrations of olopatadine in the skin have not been determined after administration via nasogastric tube (or PO) in horses, the aforementioned findings in other studies suggest that delayed distribution to the skin and sustained presence of the drug in the skin were contributing factors to the counterclockwise hysteresis pattern observed in the present study. To the authors' knowledge, there are no reports on the speed of receptor kinetics or activity of equine H_1 receptors, and the mechanisms underlying this finding remain unknown.

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The authors declare that there were no conflicts of interest.

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Footnotes

a. Wako Pure Chemical Industries, Osaka, Japan.
b. Angiocath, BD Biosciences, Franklin Lakes, NJ.
c. Mitutoyo Dejipa 700-125, Mitutoyo Corp, Kawasaki, Japan.
d. Allelock tablets, 5 mg, Kyowa Hakko Kirin, Tokyo, Japan.
e. Venoject II VP-H100K, Terumo Medical Corp, Tokyo, Japan.
f. Shimadzu LCMS-2010A mass spectrometer, Shimazu Corp, Kyoto, Japan.
g. Shimadzu prominence HPLC system, Shimazu Corp, Kyoto, Japan.
h. Oasis HLB extraction cartridge, Waters Corp, Milford, Mass.
i. Sigma-Aldrich Corp, St Louis, Mo.
j. XBridge Shield C18 column, Waters Corp, Milford, Mass.
k. Agilent GCMS system (GC:6890, MS:5973), Agilent Technologies, Santa Clara, Calif.
l. LCMSsolution, version 2.01, Shimazu Corp, Kyoto, Japan.
m. IBM SPSS Statistics, version 19, IBM Corp, Armonk, NY.
n. JMP, version 13.1.0, SAS Institute Inc, Cary, NC.

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