In vivo and in vitro effects of neostigmine on gastrointestinal tract motility of horses

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Objective—To determine the response to neostigmine of the contractile activity of the jejunal and pelvic flexure and the effects of a continuous rate infusion (CRI) of neostigmine in horses.

Animals—7 adult horses and tissue from 12 adult horses.

Procedures—A CRI of neostigmine (0.008 mg/kg/h) or placebo was administered to 6 horses in a crossover study design. Gastric emptying was evaluated by the acetaminophen test. The frequency of defecation and urination and the consistency and weight of feces were recorded throughout the experiment. The effect of neostigmine on smooth muscle contractile activity was evaluated in tissues from the jejunum and pelvic flexure. The effect of neostigmine and acetylcholine after incubation with muscarinic receptor antagonists (atropine and DAU 5884) and an acetylcholinesterase inhibitor (edrophonium) was also investigated in vitro.

Results—No difference was observed between neostigmine and placebo for time to reach peak plasma acetaminophen concentration and absorption rate constant. A CRI of neostigmine increased fecal production and frequency of urination. Neostigmine induced a dose-dependent increase of contractile amplitude in jejunum and pelvic flexure muscle strips. Incubation of muscle strips with atropine and DAU 5884 inhibited the response to acetylcholine and neostigmine. Incubation of smooth muscle strips from the jejunum with edrophonium increased the response to acetylcholine and had no effect on the response to neostigmine in vitro.


A dynamic ileus is characterized by loss of propulsive contractile activity and gastrointestinal tract coordination, leading to accumulation of fluids and formation of gas in the gastrointestinal tract, causing discomfort. The causes of adynamic ileus are multifactorial, and when it develops as a complication of gastrointestinal tract surgery, it is identified as POI. Reported conditions and factors associated with POI include systemic shock, type and location of lesion, duration of surgery and anesthesia, need and length of resection, and type of anastomosis. Recent studies found an overall prevalence of POI after colic surgery from 14% to 22%. More than 50% of fatalities after colic surgery occur in the postoperative period, and the most common reasons for death or euthanasia during the postoperative period are POI, circulatory or endotoxic shock, and persistent signs of pain. Because of the multifactorial and complex causes of ileus in horses, a variety of treatment options have been proposed, including the use of pharmacological agents to increase propulsive activity of the intestine. Prokinetic agents (cholinomimetics, adrenergic antagonists, benzamides, macrolide antimicrobials, dopamine antagonists, local anesthetics, and phenoxybenzamine) have been used in horses with variable success. Neostigmine, a parasympathomimetic agent, prolongs the activity of acetylcholine by inhibiting the acetylcholinesterase enzyme, retarding the breakdown of Alzheimer's disease.

Abbreviations

<table>
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<tr>
<th>Abbreviation</th>
<th>Description</th>
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<tr>
<td>AUC</td>
<td>Area under the curve</td>
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<td>Cmax</td>
<td>Peak plasma acetaminophen concentration</td>
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<td>CRI</td>
<td>Continuous rate infusion</td>
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<td>Ka</td>
<td>Absorption rate constant</td>
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<td>KRB</td>
<td>Modified Krebs Ringer buffer solution</td>
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<tr>
<td>POI</td>
<td>Postoperative ileus</td>
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<td>Tmax</td>
<td>Time to reach peak plasma acetaminophen concentration</td>
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acetylcholine at the synaptic junction. Neostigmine has been evaluated in vivo in the proximal and distal portions of the gastrointestinal tract of horses and ponies. Administration of a dose of neostigmine methylsulfate (0.022 mg/kg) repeated 4 times at 30-minute intervals delays gastric emptying in healthy horses. A single dose of neostigmine increases the amplitude of rhythmic contractions in isolated jejunal segments of healthy anesthetized ponies and increases myoelectrical activity of the ileum or has no effect on myoelectrical and mechanical activity of distal jejunum in vivo. In the large intestine of healthy ponies, a rhythmic contractions in isolated jejunal segments of healthy anesthetized ponies and increases myoelectrical activity of the ileum or has no effect on myoelectrical and mechanical activity of distal jejunum in vivo. Continuous rate infusion of neostigmine promotes defecation in human patients with colonic ileus; however, it fails to increase gastric emptying and enteral feed absorption in critically ill patients. Neither the in vivo effects of a CRI of neostigmine on gastric emptying and defecation frequency in clinically normal horses nor the in vitro effects of neostigmine in the gastrointestinal tract of horses have been evaluated. The objectives of the study reported here were to determine the effects of a CRI of neostigmine on gastric emptying, fecal production, defecation, and urination frequency in healthy horses in vivo and to determine the response to neostigmine of the contractile activity of equine jejunum and pelvic flexure muscle strips in vitro. We hypothesized that neostigmine would accelerate gastric emptying and defecation frequency in healthy horses and that, in vitro, it would stimulate jejunum and pelvic flexure contractility.

Materials and Methods

In vivo study—Seven adult horses (4 females and 3 geldings) from the Center for Equine Health, University of California-Davis, were used in the in vivo part of the study. Horse breeds were Quarter Horse (n = 2), Thoroughbred (1), Standardbred (1), Saddlebred (1), Morgan (1), and Paint (1). Median age and body weight were 17 years (range, 9 to 21 years) and 576 kg (range, 508 to 616 kg), respectively. All procedures were approved by the University of California Institutional Animal Care and Use Committee. The selected horses were healthy and had no recent colic episode or previous abdominal surgery.

One horse was used to determine the time needed for a CRI of neostigmine to reach a steady state. A catheter was aseptically inserted into each jugular vein, one for neostigmine administration and the other for blood collection. Neostigmine methylsulfate was administered as a CRI by use of a syringe pump to deliver 0.008 mg/kg/h for 6 hours. Venous blood (3 mL) was collected for neostigmine determination from the opposite jugular catheter into heparinized evacuated tubes before infusion (baseline) and during the infusion period at 5, 10, 20, 30, 40, and 50 minutes and at 1, 1.5, 2, 2.5, 3, 3.5, 4, 4.5, 5, and 6 hours. Immediately after collection, blood was centrifuged at 2°C for 5 minutes, and the plasma was collected and immediately frozen at –20°C and stored at –80°C on the same day.

The other 6 horses were used in a randomized crossover design with a 2-week interval between studies. Before and between studies, horses were kept in a dry (nonirrigated) pasture and were fed a mixture of 50% alfalfa hay and 50% grass hay ad libitum. Horses were moved to a stall 24 hours before the experiment. Horses were fed a mixture of alfalfa hay and grass hay at 1% of body weight twice daily while stabled. Food and water were withheld for 12 and 6 hours, respectively, before initiation of the study. Horses did not receive food or water during the duration of the experiment. A catheter was aseptically placed in each jugular vein. One catheter was used for the CRI of neostigmine or placebo and the other catheter for blood sampling and crystalloid fluid administration. Horses were sedated with 200 mg of xylazine hydrochloride, and a nasogastric tube was placed before the continuous infusion was started. Once the nasogastric tube was in the stomach, it was marked to keep the same length of tube in the stomach throughout each experiment. The nasogastric tube was left in place until the end of each study (5.5 hours). For each experiment, each horse received either a continuous IV infusion of neostigmine methylsulfate (0.008 mg/kg/h) or the same volume of saline (0.9% NaCl) solution (placebo) for 5.5 hours. During the entire experiment, horses received crystalloid fluids at a maintenance rate (2 mL/kg/h). Acetaminophen powder (20 mg/kg, mixed with 400 mL of deionized water) was administered via the nasogastric tube followed by 100 mL of deionized water 3 hours after initiation of neostigmine or placebo infusion. Venous blood (3 mL) was collected into heparinized evacuated tubes for acetaminophen determination immediately before administration (time 0; baseline) and 2, 5, 7, 10, 15, 20, 30, 45, 60, 90, 120, and 130 minutes after acetaminophen administration. Immediately after collection, blood was centrifuged at 2°C for 5 minutes, and the plasma was centrifuged at 2°C for 5 minutes and stored at –20°C and at –80°C on the same day. During the entire experiment, horses were videotaped and closely monitored for signs of abdominal pain. Physical examinations were performed (heart and respiratory rates and rectal temperature) at baseline and during the CRI at 5, 10, 20, 30, 40, and 50 minutes and at 1, 1.5, 2, 2.5, 3, 3.5, 4, 4.5, 5, and 5.5 hours. Frequencies of defecation and urination and consistency of feces were recorded, and the feces were weighed. Fecal consistency was graded by one of the authors (BM) by application of a grading system used in the hospital’s intensive care unit, where 1 = normal dry feces, 2 = soft solid feces maintaining a form, 3 = soft nonformed feces, 4 = soft nonformed feces with watery projectile diarrhea, and 5 = only watery projectile diarrhea. The feces from each defecation episode were graded, and the mean from all defecation episodes was used for statistical analysis. After each experiment, the nasogastric tube and IV catheters were removed, and horses were fed and kept overnight in the stall for observation.

Acetaminophen concentration was quantified in plasma from horses via liquid chromatography–tandem mass spectrometry after extraction cleanup with a protein precipitation procedure as described. The concentration of acetaminophen in each sample was determined by use of an internal standard method by use of peak area ratio and linear regression analysis.
The technique was optimized to provide a minimum limit of quantification of 10 ng/mL for acetaminophen. Determination of plasma neostigmine concentration was performed by high-performance liquid chromatography–tandem mass spectrometry. After extraction cleanup with a protein precipitation procedure, the concentration of neostigmine in each sample was determined by use of an internal standard (benzylidimethylphenyl ammonium) method by use of peak area ratio and linear regression analysis. The technique was optimized to provide a lower limit of quantification of 10 pg/mL, and the lower limit of detection was 5.0 pg/mL.

Gastric emptying was evaluated by the acetaminophen absorption test. Pharmacokinetic data analysis was performed with a commercial software program, and plasma acetaminophen concentration-time data were assessed by use of noncompartmental analysis modeling. Peak plasma acetaminophen concentration and T\textsubscript{max} were estimated from the data. Linear trapezoidal areas were used in calculating the plasma acetaminophen AUC, and other pharmacokinetic parameters were determined by use of standard noncompartmental equations. Individual modeling of plasma acetaminophen concentrations was performed with a 1-compartment model with first-order absorption and first-order elimination. The model was parameterized with absorption lag time, apparent volume of distribution at steady state, K\textsubscript{e}, and elimination rate constant.

**In vitro study**—Tissue was obtained from 12 adult horses with a median age of 13.6 years (range, 8 to 18 years). Tissue from 6 horses was used for dose response testing, and tissue from the other 6 horses was used to determine the effect of neostigmine and acetylcholine after incubation with muscarinic receptor antagonists and an acetylcholinesterase inhibitor. Horse breeds were Quarter Horse (n = 6), Arabian (2), Paint (1), Thoroughbred (1), Warmblood (1), and Lusitano (1). None of the horses had gastrointestinal tract disorders, and an acetylcholinesterase inhibitor. Horse breeds were Quarter Horse (n = 6), Arabian (2), Paint (1), Thoroughbred (1), Warmblood (1), and Lusitano (1). None of the horses had gastrointestinal tract disorders, or were receiving any treatment for reasons unrelated to the study. Segments from the antimesenteric border of the pelvic flexure and midportion of the jejunum (4 vascular arcades orad from the ileum) were collected immediately after euthanasia. Ingesta were removed by washing the lumen with KRB, which contained 110mM NaCl, 4.6mM KCl, 2.5mM CaCl\textsubscript{2}, 24.8mM NaHCO\textsubscript{3}, 1.2mM KH\textsubscript{2}PO\textsubscript{4}, 1.2mM MgSO\textsubscript{4}, and 5.6mM glucose and had a pH of 7.3 to 7.4 when equilibrated with 95% O\textsubscript{2} and 5% CO\textsubscript{2}. After washing, the tissue was placed in cold oxygenated KRB. All samples were tested the same day of collection as described.

Briefly, segments of tissue were pinned flat in a dissecting dish containing KRB. Full-thickness muscle strips (2 X 10 mm) were cut parallel to the circular muscle fibers, and the mucosa and submucosa were removed with the aid of a dissecting microscope. The muscle strips were suspended in organ baths containing 20 mL of continuously oxygenated KRB at 37°C. The distal end of each strip was attached to a glass hook tissue support, and the proximal end was attached to an isometric force transducer. Strips were allowed to rest without tension for 30 minutes and then stretched with the tension of 1 g followed by an additional 1 g 20 minutes later, to receive 2 g of stretch. Experiments were performed with the muscle strips under this tension. This degree of muscle tension was determined in a previous study to result in maximal active tension development in the equine circular muscle. The same tension was used for the pelvic flexure smooth muscle to maintain a constant tension in all tissues used for the dose-response testing. The KRB was changed every 30 minutes throughout the equilibration period.

Isometric force was recorded by use of force transducers connected by a transducer cable to an 8-channel polygraph chart recorder and a computer. Data were recorded and analyzed by use of a software package. Data were normalized by subtracting baseline values either before the cumulative dose responses or after incubation with the specific antagonists.

Cumulative dose responses to neostigmine methylsulfate were evaluated on midjejunum (n = 14 experimental and 14 control strips from 6 horses) and pelvic flexure (14 experimental and 14 control strips from 6 horses) muscle strips. Experimental (neostigmine) and control (vehicle) strips for each horse were run concurrently. After 90 minutes of stabilization, baseline values were recorded for 3 minutes. Cumulative concentrations of neostigmine or vehicle were added (10\textsuperscript{−9} to 10\textsuperscript{−6}M) every 3 minutes, and the contractile activity was recorded.

To determine the mechanism of action of neostigmine in equine smooth muscle, muscle strips of jejunum were incubated with a nonselective muscarinic receptor antagonist (atropine sulfate) [10\textsuperscript{−6}M] for 20 minutes; n = 14 experimental and 14 control strips

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![Image](Figure1.png)
from 6 horses), an M3 selective muscarinic receptor antagonist (DAU 5884 hydrochloride \(10^{-6}\)M) for 20 minutes; \(n = 12\) experimental and 12 control strips from 6 horses), or a reversible acetylcholinesterase inhibitor (edrophonium chloride \(10^{-4}\)M) for 3 minutes; 12 experimental and 12 control strips from 6 horses). Control strips were incubated with the same volume of vehicle. After incubation, the dose of acetylcholine (1.28 \( \times 10^{-6}\)M) or neostigmine (2.5 \( \times 10^{-6}\)M) that induced 50% of the maximum response, determined from preliminary experiments, was added to experimental and control strips, and the contractile activity was recorded for 10 minutes.

Statistical analysis—To determine whether a CRI of neostigmine had a significant (P < 0.05) effect on acetaminophen pharmacokinetic variables in vivo, the Wilcoxon rank test was used to compare Cmax, Tmax, K\(_a\), and AUC. Frequencies of defecation and urination, mean values of fecal consistency, and amount of feces produced between treatments were also analyzed by the Wilcoxon rank test. Heart rate, respiratory rate, and body temperature were compared by 2-way repeated-measures ANOVA to determine a difference between treatments.

Table 1—Amount and consistency of feces and frequency of defecation and urination in 6 horses that received a CRI of neostigmine (0.008 mg/kg/h) and saline (0.9% NaCl) solution for 5.5 hours in a crossover study design.

<table>
<thead>
<tr>
<th>Group</th>
<th>Feces (kg)</th>
<th>Feces (mean consistency)</th>
<th>Defecation (frequency)</th>
<th>Urination (frequency)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>1.5 (1–2.3)</td>
<td>1 (0–1)</td>
<td>1.5 (0–3)</td>
<td>0 (0–1)</td>
</tr>
<tr>
<td>Neostigmine</td>
<td>2.9 (1.7–8.1)*</td>
<td>1.4 (1–2.9)</td>
<td>3.5 (1–10)</td>
<td>1 (1–15)*</td>
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Values are median (range). *Significant (P < 0.05) difference between treatments.

Results

In vivo study—The plasma concentration resulting from a CRI of neostigmine (0.008 mg/kg/h) in the pilot horse was graphed (Figure 1). Neostigmine was first detected in the 10-minute sample. Neostigmine concentrations reached a steady state after 2.5 hours of infusion; the concentration remained stable for the remaining 3.5 hours of infusion.

A CRI of neostigmine (0.008 mg/kg/h) was well tolerated by 3 horses (pilot horse and 2 experimental horses). Three horses developed mild signs of colic (pawing) at 1.5, 2, and 2.5 hours of the neostigmine infusion, and the signs lasted for < 15 minutes. The signs of discomfort resolved with no intervention, and the horses appeared comfortable for the rest of the infusion period. One horse developed more severe signs of colic after 5 hours of the neostigmine infusion. The horse was pawing and sweating and tried to lie down. The horse was walked in the stall for 20 minutes. Neostigmine infusion was stopped 10 minutes prior to the scheduled end of the experiment. After the last blood sample was collected (5.5 hours), the horse received an IV dose of flunixin meglumine (1 mg/kg). The
coli signs resolved within minutes. Data from the horse were included in the analysis. No differences for heart and respiratory rates and body temperature were observed between treatments. Median and range values for amount of feces, consistency of feces, and frequencies of defecation and urination were calculated (Table 1). There was no difference for the consistency of feces ($P = 0.068$) or defecation frequency ($P = 0.066$) between treatments. The amount of feces passed was higher ($P = 0.046$) and urination was more frequent ($P = 0.039$) with the neostigmine than with the control treatment.

Curve-fitted data for each treatment group were determined (Figure 2), and pharmacokinetic variables of the absorption of acetaminophen for both groups were calculated (Table 2). The pharmacokinetic values for acetaminophen absorption AUC and Cmax were higher with the neostigmine treatment than the control infusion. In 1 horse from the control group, the acetaminophen concentrations did not fit the absorption patterns of the parameter estimation software, and $K_a$ could not be determined.

In vitro study—Neostigmine induced a significant dose-dependent increase from baseline values in contractile amplitude of the midjejunum from $10^{-9}$M (mean ± SEM, $31 ± 27$ g/cm$^2$) to $10^{-4}$M ($493 ± 152$ g/cm$^2$). Similar significant increases were evident for the pelvic flexure from $10^{-5}$M ($228 ± 143$ g/cm$^2$) to $10^{-4}$M ($508 ± 175$ g/cm$^2$; Figure 3).

Incubation of muscle strips from the jejunum with atropine sulfate and DAU 5884 hydrochloride inhibited the response to acetylcholine and neostigmine (Figure 4). Incubation of muscle strips from the jejunum with edrophonium chloride increased the response to acetylcholine and had no effect on the response to neostigmine (Figure 5).

Discussion

Despite advances in understanding of intestinal injury and repair, POI remains an important cause of morbidity and death in horses. Because of multifactorial causes, a reduced number of available prokinetic agents, and a limited number of specific studies in
horses, the efficacy of prokinetic agents and the guidelines for their use in the perioperative treatment of horses considered at risk of developing ileus are unclear. A survey on the use of prokinetic agents in horses with gastrointestinal tract injury revealed that neostigmine is the first choice by veterinarians for lesions involving the large intestine. Reported doses of neostigmine for use in horses are 0.02 to 0.04 mg/kg, IV or SC, at intervals determined by the horse's response. However, similar doses have induced signs of abdominal discomfort in ponies in an experimental setting. Neostigmine administration in humans has been associated with abdominal cramps, diarrhea, salivation, bradycardia, hypotension, and bronchoconstriction. To decrease potential adverse effects and to maintain stable plasma concentrations, some agents are used as continuous infusions. A CRI prevents the sudden peaks and valleys associated with intermittent bolus administrations. Medications currently given as CRIs in equine postoperative colic cases include lidocaine and metoclopramide. Because no pharmacokinetic studies of neostigmine in horses have been reported, for the present study, the mean of the CRI dosages reported for human patients (0.008 mg/kg/h) was selected. To determine whether the 0.008 mg/kg/h dosage was safe to administer to horses and to determine the time required for a CRI of neostigmine to reach stable blood concentration, a pilot horse received a CRI of neostigmine for 6 hours. Plasma neostigmine concentrations were first detected 10 minutes after initiation of the CRI. Stable plasma concentrations of neostigmine were detected by 2.5 hours of infusion, and these concentrations remained stable to the end of infusion (6-hour sample). To obtain steady state concentrations more quickly, the administration of a bolus at the beginning of the infusion or administration of double the infusion rate for the first half-life of the drug is recommended. However, considering that signs of abdominal pain may be induced by neostigmine administration, use of a bolus is not recommended.

Neostigmine was tolerated by 6 of the 7 horses that received a CRI of neostigmine. However, mild signs of discomfort were observed for a short period (< 15 minutes) in 3 horses, mainly before passing feces, and the horses had no signs of discomfort for the rest of the infusion without any treatment or discontinuation of the drug. However, more severe signs of pain were observed in 1 horse, for which the infusion needed to be discontinued and the horse consequently treated with analgesics. Administration of neostigmine as in this study should be used with caution in clinical cases because horses at risk of or with POI may already have visceral pain. Agents with a short elimination half-life benefit from a CRI because therapeutic concentrations are easier to maintain. In addition, the rate of infusion can easily be changed to induce the desired effect. After stopping the infusion, the amount or concentration of drug in the body decreases by one-half for each half-life. Therefore, discontinuation of the infusion is all that may be needed to stop adverse effects.

Neostigmine increases progressive motility of the large intestine and induces defecation in healthy ponies. Administration of neostigmine as a CRI in horses at risk of or with POI may already have visceral pain. Agents with a short elimination half-life benefit from a CRI because therapeutic concentrations are easier to maintain. In addition, the rate of infusion can easily be changed to induce the desired effect. After stopping the infusion, the amount or concentration of drug in the body decreases by one-half for each half-life. Therefore, discontinuation of the infusion is all that may be needed to stop adverse effects.
Figure 5—Mean ± SEM contractile force detected after addition of a half–maximal-effective-concentration dose of neostigmine (2.5 × 10⁻⁶M) or acetylcholine (1.28 × 10⁻⁶M) to strips (0.5 cm wide) of circular smooth muscle obtained from the midportion of the jejunum of 6 horses. Strips were incubated for 3 minutes with edrophonium chloride or vehicle. After neostigmine or acetylcholine administration, the contractile activity was recorded for 10 minutes. See Figures 3 and 4 for remainder of key.

The acetaminophen absorption test has been used in horses and other species to evaluate the effect of pharmacological agents and nasogastric intubation on gastric emptying of liquids.3,26–29,43–46 Parameters used to evaluate gastric emptying by the acetaminophen absorption test included Cmax, Tmax, AUC, and Ka. However, 2 studies27,63 in horses comparing gastric emptying evaluated by nuclear scintigraphy (considered to be the gold standard) with the acetaminophen absorption test found that only Tmax and Ka correlate with the half-life of liquid-phase gastric emptying. No differences were detected for Tmax and Ka between treatments in the present study, indicating that a CRI of neostigmine neither increased nor decreased gastric emptying of fluids. Studies in horses using the acetaminophen absorption test found that agents known to decrease gastric emptying (atropine and xylazine) had an effect on Cmax or AUC.36,60 A common value used to evaluate gastric emptying by use of by nuclear scintigraphy is the half-life of liquid-phase gastric emptying. However, a recent report45 indicates that such a simple parameter would not be sufficient to compare the curves from different methods and instead that study compared the 3-quartile degrees of retention (75%, 50%, and 25%). In humans, Cmax, Tmax, and AUC from the acetaminophen absorption test have been compared with scintigraphy and evaluated in a systematic literature review.47 Of the 4 studies comparing Cmax with scintigraphy, 2 found satisfactory correlation and 2 found poor correlation. Of the 7 studies comparing AUC with scintigraphy, 3 found good correlation, 2 found moderate correlation, and 2 found poor correlation. Of the 5 studies comparing Tmax with scintigraphy, 3 found good correlation and 2 found poor correlation. If Cmax and AUC are indicators of gastric emptying in horses as has been suggested in other species, then a CRI of neostigmine may be beneficial for the prevention or treatment of horses with P01.

A previous study18 revealed that SC administration of neostigmine (0.022 mg/kg, repeated 4 times every 30 minutes) decreases gastric emptying as evaluated by the passage of markers. However, that study18 used 4 bolus doses over 2 hours; therefore, the total dose was approximately 10 times the dose administered in this study. It is possible that the administration of neostigmine boluses at high doses induces nonpropulsive contractions, causing a delay of gastric emptying, and that lower concentrations over longer time (CRI) may result in a more normal function by maintaining more constant blood concentrations. In addition, differences in the routes of administration (SC vs IV), experimental methodology (transit of plastic markers vs acetaminophen test), materials of gastric emptying evaluated (plastic beads vs liquid), and types of administration (boluses vs CRI) may also be responsible for the differences observed between studies. Because of the report-
ed adverse effects on gastric outflow, the use of neostigmine has been considered to be contraindicated in most equine postoperative motility disorders. The present study found that a CRI of neostigmine did not decrease gastric emptying, as measured by Tmax and Cmax, as has been reported. Additional studies in live horses evaluating other doses of neostigmine administered as a CRI and the use of a CRI of neostigmine to prevent or treat POI are warranted. It will also be of interest to determine the relevance of finding significant differences in Cmax and AUC when the acetaminophen test is used to evaluate gastric emptying.

An effect of an IV overdose of pentobarbital (for euthanasia) on gastrointestinal tract motility in the present in vitro study cannot be excluded. However, no studies have been performed in horses to determine the effect of different euthanasia methods on smooth muscle contractility. The same method of euthanasia has been used in our laboratory and other institutions in similar equine in vitro studies. Euthanasia by IV pentobarbital overdose in rabbits has no effect on ileal or aortic contractility or on aortic prostaglandin production, compared with decapitation. The report of that study concluded that pentobarbital overdose is the euthanasia technique that induces the least clinically relevant alterations of vascular arachidonic acid metabolism and vascular and intestinal smooth muscle contractility, compared with decapitation alone. Therefore, we believe pentobarbital overdose is an acceptable method of euthanasia for these types of studies.

During peristalsis, circular smooth muscle contractions take place in coordination with shortening of the longitudinal muscle, causing transit of ingesta. In the present study, only the in vitro effects of neostigmine on the circular smooth muscle layer were evaluated. However, other studies have found different responses between circular and longitudinal smooth muscle layers. Evaluating drug responses of different muscle layers under different stimuli may improve the ability to correlate in vitro results with physiologic and pathologic responses. Therefore, when possible, evaluation of both circular and longitudinal muscle layers of the intestine is recommended.

Neostigmine administration induced a concentration-dependent increase in the contractile activity of the circular muscle strips of midjejunum and pelvic flexure in the horses of the present study. The contractile response of muscle strips of the midjejunum to half maximal effective concentrations of neostigmine was similar to the response induced by acetylcholine. To try to determine the mechanism of action of neostigmine, jejunal muscle strips were incubated with atropine, a nonselective competitive antagonist for the muscarinic receptor types M1 to M5, and with DAU 5884, a selective muscarinic M3 receptor antagonist. Both neostigmine and acetylcholine failed to elicit a contractile response from jejunal muscle strips incubated with the antagonist. The fact that both antagonists abrogated the effects of neostigmine indicated that neostigmine, either directly or via acetylcholine, acts on the M3 receptor. To determine whether the main mechanism of action of neostigmine was inhibiting the cholinesterase enzyme, muscle strips were incubated with edrophonium chloride, a reversible acetylcholinesterase inhibitor. Incubation of muscle strips with edrophonium increased the response of the smooth muscle to acetylcholine, compared with baseline values and control strips, indicating that the inhibition of acetylcholinesterase increased the availability of acetylcholine. A high concentration (10−4 M) of edrophonium was used in the tissue bath in an attempt to completely block acetylcholinesterase activity. By blocking acetylcholinesterase activity, prevention of the contractile response induced by neostigmine was expected. However, muscle strips incubated with edrophonium responded to neostigmine. Edrophonium reduced the contractile response to neostigmine from 391 to 170 g/cm², compared with control strips; however, the reduction did not reach significance. Similarly, a previous study found that incubation of guinea pig ileal muscle strips with dylos, an irreversible acetylcholinesterase inhibitor, does not affect the contractile response to neostigmine. It is unknown whether edrophonium completely inhibited the acetylcholinesterase in the present study. In addition, edrophonium is a reversible inhibitor, and although a short incubation time (3 minutes) was used, it is not known what percentage of inhibition was still present at the time of neostigmine stimulation. If edrophonium only partially blocked acetylcholinesterase activity, then addition of neostigmine would still have induced an increase in contractile activity similar to that observed in this study. Therefore, additional experiments to determine whether neostigmine has other mechanisms of action in addition to increasing acetylcholinesterase activity or concentration are warranted.

Neostigmine stimulated in vitro contractile activity of the jejunum and pelvic flexure muscle strips. A CRI of neostigmine did not decrease gastric emptying as described elsewhere. A CRI of neostigmine was safe to administer and increased the amount of feces produced and frequency of urination in healthy horses.

References


