Evaluation of the effect of ranitidine on gastroduodenal contractile activity and gastric emptying in horses

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Objective—To determine the effect of ranitidine on gastric emptying in horses.

Animals—11 adult horses.

Procedures—In vitro, isolated muscle strips from the pyloric antrum and duodenum of 5 horses were suspended in baths and attached to isometric force transducers. Once stable spontaneous contractions were observed, ranitidine or diluent was added at cumulative increasing concentrations. Isometric stress responses were compared. In vivo, 6 horses were assigned to a group in a prospective randomized crossover study design with a washout period of 2 weeks between trials. Ranitidine (2.2 mg/kg) or saline (0.9% NaCl) solution was administered IV, and 15 minutes later, acetaminophen (20 mg/kg), diluted in 400 mL of water, was administered via nasogastric tube to evaluate the liquid phase of gastric emptying. Serum acetaminophen concentration was measured at several time points for 3 hours by use of liquid chromatography tandem mass spectrometry. Frequency of defecation was recorded during the 3 hours of the study.

Results—Ranitidine increased the contractile activity of the pyloric antrum smooth muscle at a concentration of $10^{-4}$M. No significant effect of ranitidine on plasma kinetics of acetaminophen was identified. Frequency of defecation did not differ between groups.

Conclusions and Clinical Relevance—Ranitidine did increase gastric motility in vitro, but no effect on liquid phase gastric emptying was identified in healthy horses by use of the acetaminophen absorption model. Results do not support the use of ranitidine to promote gastric emptying. (Am J Vet Res 2008;69:1153–1157)

Abstract

Abnormal gastric emptying and resulting gastric distention may be factors in the pathogenesis of gastric ulceration in horses and are important features of postoperative ileus and duodenitis-proximal jejunitis. The resulting gastric distention has been associated with colic, prolonged ileus via gastrointestinal reflex inhibition, and the risk of catastrophic gastric rupture.

Ranitidine is a histamine type 2 receptor antagonist used to inhibit gastric acid secretion in various species, including humans, dogs, and horses. Ranitidine also acts as an acetylcholinesterase inhibitor, stimulating gastrointestinal motility by increasing the amount of acetylcholine available to bind smooth muscle muscarinic cholinergic receptors, and is one of the accepted treatments for gastric motility disorders in small animals. In vitro studies reveal that ranitidine increases gastric antrum motility in guinea pigs. However, results of in vivo studies differ; some studies reveal stimulation of gastric emptying in laboratory animals, dogs, and humans, others suggest that ranitidine has no effect or even causes a delay in gastric emptying in humans.

A recent report of clinical cases of colic at a referral hospital indicated an overall prevalence of gastric ulceration of 49%. Ranitidine is one of the more commonly used medications for the treatment or prevention of gastric ulceration in horses. Agents such as ranitidine or omeprazole are commonly added to the treatment of horses with abdominal pain. Ranitidine is one of the few antiulcer medications available in a parenteral formulation, making it particularly useful in treatment and prevention of gastric ulcers in horses with postoperative ileus or duodenitis-proximal jejunitis.

Abbreviations

- **AUC**: Area under the curve
- **$C_{\text{max}}$**: Maximal concentration
- **$K_a$**: Absorption rate constant
- **KRB**: Krebs Ringer’s buffer
- **LC-MS-MS**: Liquid chromatography-mass spectrometry-mass spectrometry
- **$T_{\text{max}}$**: Time of maximal concentration
Six healthy geldings (4 Thoroughbreds, 1 American Quarter Horse, and 1 Paint Horse) that were 11.6 ± 6.7 (mean ± SD) years of age with body weights of 535 ± 34 (mean ± SD) kg from the Center for Equine Health, University of California, Davis, were used for the in vivo study. None of the horses had clinical signs of gastrointestinal tract disease or a history of colic surgery.

Experimental protocol—The 6 horses were studied on 2 occasions with 2 weeks between experiments in a crossover study design. Before and between experiments, the horses were free in pasture and were also fed alfalfa hay. Horses were moved to 4-m stalls 48 hours before the experiments; food and water were withheld for 18 and 6 hours, respectively. Horses were restrained with a halter and lead rope. No sedatives were used. A catheter was placed in the left jugular vein for medication administration and blood sampling. For each experiment, each horse was randomly assigned to receive treatment (ranitidine [2.2 mg/kg], dilaun 0.5 mg/kg, distilled in 60 mL of saline [0.9% NaCl] solution, injected IV over 5 minutes) or a placebo (60 mL of saline solution, injected IV).

A nasogastric tube was placed, and acetaminophen (20 mg/kg, mixed with 400 mL of water) was administered via the nasogastric tube (followed by 100 mL of water) 15 minutes after ranitidine or placebo administration. Venous blood samples were collected at baseline (time 0) and 5, 10, 15, 20, 30, 45, 60, 90, 120, 150, and 180 minutes after acetaminophen administration. Signs of abdominal pain and fecal output (number of defecations) were recorded for 3 hours after acetaminophen administration.

Sample analysis—Analytical reference standards of acetaminophen and d4-acetaminophen were commercially obtained.1 Standard solutions of acetaminophen and d4-acetaminophen were prepared in methanol. Acetaminophen was quantified in plasma from horses via LC-MS-MS after extraction cleanup with a protein precipitation procedure. The extracts of all samples were dissolved in 100 µL of the mobile phase prior to analysis. Quantitative analyses were performed on a triple quadrupole mass spectrometer equipped with a liquid
chromatography system. Separation was performed on a carbon 18 column (100 mm; internal diameter, 2.0 mm; particle size, 3 mm) with a linear gradient of acetonitrile and water and constant 0.2% formic acid. The acetonitrile concentration was held at 2.0% for 1.5 minutes, increased from 2% to 50% over 2.5 minutes, and increased from 50% to 90% over 2.0 minutes. Detection and quantification was performed by use of selective reaction monitoring of LC-MS-MS transitions for the initial product ions for acetaminophen (mass-to-charge ratio, 152.0). 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.

The LC-MS-MS calibration curve for acetaminophen was quadratic ($R^2 > 0.99$) in the range of 10 to 30,000 ng/mL. The overall accuracy and precision of the assay in equine plasma measured by use of quality-control samples ($n = 6$) at 500 ng/mL were 93.0% and 9.6%, respectively.

Data analysis—Pharmacokinetic data analysis was performed with a commercial software program, and plasma acetaminophen concentration-time data were assessed by use of noncompartmental analysis and compartmental modeling. Plasma acetaminophen $C_{\text{max}}$ and $T_{\text{max}}$ 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 ($\pm$ lag time) and first-order elimination. The model was parameterized with absorption lag time, apparent volume of distribution at steady state, absorption rate constant $K_{\text{a}}$, and elimination rate constant. If the model fit poorly and there was not apparent lag time by visual inspection, absorption lag time was fixed at zero and fitting was repeated, but most of the plasma acetaminophen concentration profiles required an absorption lag time.

Statistical analysis—To determine whether ranitidine had an effect on the muscle strips, the amplitude of the contractions in response to increasing doses were compared by use of 2-way repeated-measures ANOVA. To assess whether ranitidine administration had a significant effect on acetaminophen pharmacokinetic variables, the Wilcoxon rank test was used to compare $C_{\text{max}}$, $T_{\text{max}}$, $K_{\text{a}}$, and AUC. All data are presented as mean ± SD. To assess the effect of ranitidine on the frequency of defecation, a $\chi^2$ test was applied. For all tests, a statistical computer program was used and significance was set at $P < 0.05$.

Figure 1—Effects of ranitidine (rectangles) or control solution (diamonds) on isolated strips of circular smooth muscle obtained from the proximal portion of the duodenum (A), from the distal portion of the pyloric antrum (B), on isolated strips of longitudinal smooth muscle obtained from the proximal portion of the duodenum (C), and from the distal portion of the pyloric antrum (D). Values are mean ± SEM contractile force detected after addition of increasing concentrations of ranitidine ($10^{-7}$ to $10^{-4}$ M) for 20 muscle strips obtained from 5 horses. *Significantly ($P < 0.05$) different from baseline value.
Results

In the in vivo study, ranitidine-treated strips contracted with more intensity than the control strips, although the difference was significant (P = 0.044) only for the longitudinal fibers of the pyloric antrum at 10⁻⁶M concentration of ranitidine (Figure 1). In the in vivo study, none of the horses had any signs of abdominal pain during the experiment. Mean ± SD number of defecations within the 3 hours after treatment was 1.5 ± 1.05 in the treated group and 0.67 ± 0.82 in the control group. This difference was not significant (P = 0.09). 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 1); no significant differences between groups were detected.

Discussion

Ranitidine increased contractility of the longitudinal smooth muscle of the antrum of the gastric pylorus in vitro, but no significant increase in the rate of gastric emptying in healthy unfed horses was evident by use of the acetaminophen absorption model. Several factors may account for the disparity between what was observed in vitro (ranitidine increased gastric smooth muscle motility) and in vivo (no significant effect on gastric emptying was detected). Ranitidine was administered over 5 minutes because rapid administration of fluid. However, because the study was performed on horses with postoperative ileus or duodenitis-proximal jejunitis, such as endotoxemia, peritonitis, electrolyte imbalance, and intestinal distention and inflammation, could not be assessed and may have resulted in a different response. A study that uses an experimental model of ileus such as an endotoxin challenge may allow a more accurate evaluation of the clinically applicable effect. In a similar experimental study, cisapride did not increase gastric emptying in normal unfed horses but increased it significantly in endotoxin-challenged horses. Some studies in humans found that ranitidine decreased gastric emptying. If this was true in horses, the use of ranitidine in horses with postoperative ileus or duodenitis-proximal jejunitis would not be recommended. In the present study, a different study indicated that the maximal concentration of ranitidine reached in vivo via IV injection of a 2.2 mg/kg dose was approximately 5,175 ng/mL, which corresponds to 1.475 x10⁻⁶M. This concentration was maintained only for a short time after IV injection and was substantially lower than the concentration that was effective in vivo (10⁻⁶M). Furthermore, administration of acetaminophen to the horses in the present study was performed 15 minutes after treatment with ranitidine or placebo, a time at which the plasma concentration of ranitidine would have been even lower. Thus, higher concentrations may be needed in vivo to reproduce the beneficial effect observed in vitro. There was a limited number of horses in the in vivo component of the study, and variability between the horses was high; therefore, a greater number of horses may result in more accurate representation of the effect of the drug in vivo.

The model for investigating the in vivo component of the study may have been a limiting factor in assessing the clinical application of ranitidine’s effect on gastric emptying. Acetaminophen absorption has been used to evaluate liquid-phase gastric emptying in many species, including cattle and horses, for which it has been validated against the gold standard method of nuclear scintigraphy.

Liquid-phase gastric emptying was evaluated in this study because the population of interest (horses with postoperative ileus or duodenitis-proximal jejunitis) typically has a gastric content composed mainly of fluid. However, because the study was performed on unfed but clinically normal horses, the effects of ranitidine in association with other pathophysiological components of postoperative ileus or duodenitis-proximal jejunitis, such as endotoxemia, peritonitis, electrolyte imbalance, and intestinal distention and inflammation, could not be assessed and may have resulted in a different response.

Table 1—Pharmacokinetic parameters (mean ± SD) for acetaminophen (20 mg/kg, PO) in 6 horses that received ranitidine (2.2 mg/kg, IV) or saline (0.9% NaCl) solution (control).

<table>
<thead>
<tr>
<th>Value</th>
<th>Control Mean ± SD</th>
<th>Ranitidine Mean ± SD</th>
<th>Wilcoxon P value</th>
</tr>
</thead>
<tbody>
<tr>
<td>K (1/min)</td>
<td>0.11 ± 0.08</td>
<td>0.23 ± 0.13</td>
<td>0.173</td>
</tr>
<tr>
<td>t1/2 (min)</td>
<td>50.83 ± 43.41</td>
<td>30 ± 13.78</td>
<td>0.465</td>
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<tr>
<td>Cmax (ng/mL)</td>
<td>18.18 ± 8.49</td>
<td>20.13 ± 4.26</td>
<td>0.753</td>
</tr>
<tr>
<td>AUC∞ (min*ng/mL)</td>
<td>1,788.01 ± 818.93</td>
<td>1,936.57 ± 419.87</td>
<td>0.600</td>
</tr>
</tbody>
</table>

Figure 2—Curve-fitted data of plasma acetaminophen concentration (mean ± SE values) for 6 horses administered acetaminophen at 20 mg/kg and ranitidine at 2.2 mg/kg (rectangles) or saline (0.9% NaCl) solution (diamonds).
ranitidine did not have a significant inhibitory effect on gastric emptying and thus can continue to be safely used as a component of a treatment program for horses with colic to treat and prevent ulceration.

Although it was not significant, 5 of the 6 horses passed feces more frequently during the 3 hours after ranitidine administration, compared with the control treatment. We believe that this observation warrants further study to determine whether ranitidine has a prokinetic effect in the distal portion of the intestinal tract.

Ranitidine had no significant effect on gastric emptying in vivo, and an increase in smooth muscle contractions in vitro was identified but only for the emptying in vivo, and an increase in smooth muscle effect in the distal portion of the intestinal tract.

Nitidine administration, compared with the control treatment, resulted in more frequent passage of feces during the 3 hours after ranitidine, compared with the control treatment. It can continue to be safely used as a component of a treatment program for horses with colic to treat and prevent ulceration.

Although it was not significant, 5 of the 6 horses passed feces more frequently during the 3 hours after ranitidine administration, compared with the control treatment. We believe that this observation warrants further study to determine whether ranitidine has a prokinetic effect in the distal portion of the intestinal tract.

References