Yohimbine ameliorates the effects of endotoxin on gastric emptying of the liquid marker acetaminophen in horses

Sam D. Meisler, Thomas J. Doherty, Frank M. Andrews, Daniel Osborne, Donita L. Frazier

Abstract

The effect of yohimbine pretreatment on gastric emptying of a liquid marker in horses was evaluated by measuring serum concentrations of acetaminophen. Gastric emptying was determined in normal, fasted horses, in horses given endotoxin (*E. coli* 055 B5; 0.2 μg/kg) intravenously, and in horses given yohimbine (0.25 mg/kg, IV, over 30 minutes) plus endotoxin. Acetaminophen (20 mg/kg) was given by stomach tube 15 minutes after the endotoxin infusion. Blood samples for acetaminophen analysis were collected, and time to reach the peak serum concentration (T_{max}), the maximum serum concentration (C_{max}) and the area under the acetaminophen serum concentration versus time curve (AUC) were determined for each treatment group. Endotoxin significantly increased T_{max}, indicating a profound delay in gastric emptying and yohimbine pretreatment significantly ($P \leq 0.05$) prevented this effect.

Résumé

L'effet d'un pré-traitement de yohimbine sur la vidange gastrique d'un marqueur liquide chez les chevaux fut évalué en mesurant les concentrations sériques d'acétaminophène (AP). La vidange gastrique fut déterminée chez des chevaux témoins, chez des chevaux à jeun, chez des chevaux ayant reçu de l'endotoxine de E. coli *O55 B5 (0,2 μg/kg) par voie intraveineuse (IV), et chez des chevaux ayant reçu de la yohimbine IV (0,25 mg/kg sur une période de 30 min) et de l'endotoxine. L'AP (20 mg/kg) fut administrée via un tube stomacal 15 min après l'infusion d'endotoxine. Des échantillons sanguins pour analyse d'AP furent prélevés afin de déterminer pour chacun des groupes le temps requis pour atteindre le pic de la concentration sérique (T_{max}), la concentration sérique maximale (C_{max}) ainsi que la surface sous la courbe de la concentration sérique d'AP en fonction du temps (AUC). L'endotoxine a augmenté de façon significative le T_{max} indiquant ainsi un retard marqué de la vidange gastrique. Cet effet fut contrecarré de manière significative ($P \leq 0,05$) par un pré-traitement de yohimbine.*

(Traduit par docteur Serge Messier)

Introduction

Endotoxemia is a potential complication in horses undergoing abdominal surgery for intestinal problems (1). The clinical signs of endotoxemia in horses are diverse and dose-related. When administered experimentally, endotoxin has a dose-dependent inhibitory effect on gastric and small intestinal activity (2), and significantly delays gastric emptying of a liquid marker (3). A number of drug classes have proven to be effective in blocking either the systemic or gastrointestinal effects of endotoxin. The gastric stasis (3) and systemic (4) actions of endotoxin can be decreased significantly by pretreatment with non-steroidal anti-inflammatory drugs (NSAIDs). The effects of endotoxin on gastric emptying of a liquid marker were ameliorated by prior treatment with phenylbutazone (3), cisapride (5), and metoclopramide (6).

Yohimbine, an alpha-2 (α_2) antagonist, had some beneficial effects in the treatment of trauma-induced ileus in horses (7), and decreased cecal hypoperfusion and ileus following endotoxin administration (8). These findings indicate that α_2 adrenergic receptors are involved in mediating the intestinal actions of endotoxin.

The goal of this study was to evaluate the effect of yohimbine on gastric emptying of a liquid marker in endotoxin-treated horses.

Materials and methods

Animals

Twenty-one, adult (4 y to 15 y), mixed-breed mares (332 kg to 580 kg), were used in the study. The mares had not previously been used in any studies involving endotoxin. The horses' health status was evaluated by physical examination, complete blood count, and serum biochemical profile. Vaccination and deworming programs were up to date. Mares were housed individually in box stalls and fed grass hay and water. Food was withheld for 24 h, and water for 2 h, prior to each experiment. On the morning of the experiment, a jugular catheter was placed for the administration of

Department of Large Animal Clinical Sciences (Meisler, Doherty, Andrews, Osborne), Department of Comparative Medicine (Frazier), The University of Tennessee, College of Veterinary Medicine, PO Box 1071, Knoxville, Tennessee 37901-1071 USA.

Address correspondence and reprint requests to Dr. T.J. Doherty, fax: (865) 974-5773, e-mail: tdoherty@utk.edu.

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Table I. Group mean values ± standard error of serum acetaminophen T_{max}, C_{max}, and AUC after intragastric administration to horses treated with IV saline, endotoxin, and yohimbine plus endotoxin

Treatment	T_{max} (min)	C_{max} (mg/dL)	AUC (mg.min/dL)
Saline	35 ± 5	4.9 ± 0.3	708 ± 45
Endo	135.4 ± 30[a]	1.6 ± 0.4[a]	271 ± 86[a]
Yoh + Endo	35.6 ± 5[b]	4.2 ± 0.2[b]	630 ± 49[b]

AUC — area under the serum acetaminophen vs time curve (time = 0 → 240); C_{max} — maximum serum concentration of acetaminophen; Endo — endotoxin; T_{max} — time to reach maximum serum acetaminophen concentration; Yoh — yohimbine.

[a] Significantly different than control group ($P < 0.05$)

[b] Significantly different than endotoxin group ($P < 0.05$)

intravenous solutions. The study protocol was approved by the University of Tennessee Animal Care and Use Committee.

Experimental protocol

Gastric emptying was assessed using acetaminophen as a liquid marker (5,6). Twenty-one mares were randomly assigned to one of 3 groups: Group 1, control ($n = 7$); Group 2, endotoxin ($n = 7$); and Group 3, yohimbine plus endotoxin ($n = 7$). Mares were pretreated with either 1 L normal saline (Groups 1 and 2) or yohimbine (Sigma Chemical Company, St. Louis, Missouri, USA), 0.25 mg/kg, made up to 1 L in normal saline (Group 3). The pretreatment was administered over 30 min. Five minutes after pretreatment, an IV infusion of either 1 L of saline (Group 1) or endotoxin (*E. coli* 055 B5 TCA Ext.; Sigma Chemical Company), 0.2 μg/kg in 1 L saline (Groups 2 and 3), was administered over 15 min.

Acetaminophen (Sigma Chemical Company), 20 mg/kg in 1 L of water at 25°C, was administered by nasogastric tube 15 min after completion of the endotoxin or saline infusion. Rectal temperatures and heart rates were recorded at baseline, and at 30 min intervals for the 240-minute period following the endotoxin infusion.

At the conclusion of the experiment, Group 2 and 3 mares were given flunixin meglumine (Schering-Plough Animal Health Corp., Kenilworth, New Jersey, USA), 1.1 mg/kg, IV, to ameliorate sequelae from endotoxin.

Serum samples and acetaminophen analysis

Venous blood, for acetaminophen analysis, was collected at baseline (0) and 5, 10, 15, 30, 45, 60, 75, 90, 120, 180, and 240 min after acetaminophen administration. Serum samples were stored at −20°C prior to analysis. A colorimetric assay (Sigma Chemical Company) was used to measure acetaminophen. A standard curve was constructed using concentrations of acetaminophen between 0.0 and 6 mg/dL in equine serum. Absorbance in serum samples from experimental horses was compared to absorbance in serum standards containing known concentrations of acetaminophen. The maximum inter- and intra-assay coefficients of variation for all acetaminophen standard concentrations tested (0.25, 0.5, 1, 1.5, 2, 4, 6 mg/dL) was less than 7%. The sensitivity of the assay was 0.2 mg/dL. Samples were assayed in duplicate.

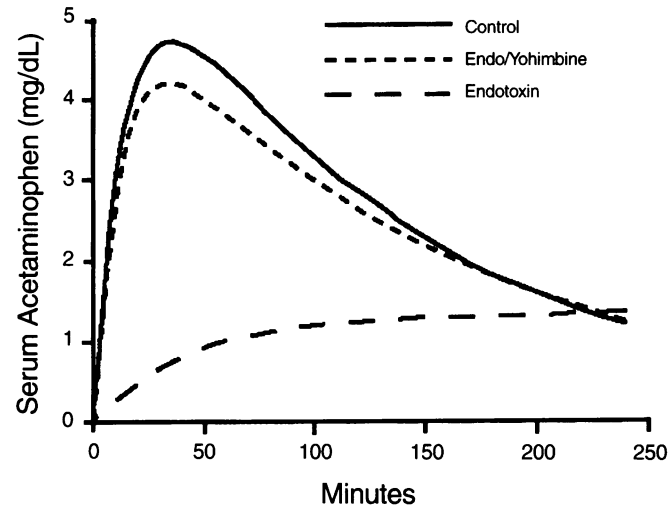

Figure 1. Effects of IV administrative of saline, endotoxin, and yohimbine plus endotoxin on serum acetaminophen concentrations vs time curves in horses. The curves were generated using data from all horses in each treatment group, and fitted using a commercially available program.

Pharmacokinetic and statistical analysis

A curve of the serum acetaminophen concentration versus time was generated for each horse using a commercially available, nonlinear, curve-fitting program (PK Analyst; MicroMath Scientific Software, Salt Lake City, Utah, USA). The time to reach maximum serum acetaminophen concentration (T_{max}), and the maximum concentration (C_{max}), were determined from the concentration versus time curve. The area under the curve (AUC), from time 0 to 240 min, was determined using the trapezoidal method. The mean and standard error values were determined for each treatment group. Differences between T_{max}, C_{max}, and AUC for each treatment group were compared using analysis of variance (Statistical Analysis Systems, Cary, North Carolina, USA). For those parameters that were significantly different ($P \leq 0.05$), least significant different (LSD) *t*-tests were used to compare each treatment group to the control group, and the endotoxin group to the groups given yohimbine plus endotoxin.

Results

Endotoxin administration delayed gastric emptying ($P \leq 0.05$), as determined by an increase in T_{max} in Group 2 compared with control Group 1 (Table I; Figure 1). The C_{max} and AUC were significantly decreased in endotoxin-treated horses (Group 2) when compared with the control (Group 1), indicating a decrease in the cumulative absorption of acetaminophen following endotoxin administration.

Clinical signs of endotoxemia developed within 60 min of endotoxin administration to Group 2 mares. Four mares became depressed with tachycardia (maximum 76 beats/min). Five mares had muscle fasciculations. All became febrile (maximum temperature 39.5°C). Four had loose feces, and 3 of these had evidence of abdominal pain, as demonstrated by pawing, sternal recumbency, and looking at their flanks. Clinical signs resolved by the end of the study, and all were keen to eat as soon as hay was offered.

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Yohimbine effectively blocked the action of endotoxin on gastric emptying, as demonstrated by a significant decrease in T_{max}. There was no difference in T_{max} between endotoxin-yohimbine treatment (Group 3) and the control (Group 1). In addition, C_{max} and the AUC were significantly increased compared to Group 2. For Group 3, C_{max} and the AUC did not differ from the controls (Group 1). Three mares in Group 3 became mildly depressed following endotoxin administration. Four had transient tachycardia (60 to 72 beats/min). Three had transient colic and 5 had loose feces. One mare had muscle fasciculations. Rectal temperature was increased in 6 mares (maximum 39.1°C).

Discussion

The administration of endotoxin significantly prolonged T_{max}, inferring an inhibition of gastric emptying. These results are consistent with previous endotoxin studies in equidae. In adult horses, endotoxin significantly delayed gastric emptying of a liquid marker, and this was ameliorated by prior treatment with phenylbutazone (3), cisapride (5), and metoclopramide (6). The dose of endotoxin used in this study was based on previous studies on gastric emptying in horses (3,5,6). In those studies, endotoxin at 0.2 μg/kg consistently delayed gastric emptying and produced mild signs of endotoxemia.

In this study, yohimbine was highly effective in blocking the gastrointestinal effects of endotoxin, as shown by a decrease in T_{max} in Group 3 mares when compared with Group 2 mares. C_{max} and AUC also increased, and this may be due to improved intestinal blood flow facilitating absorption. Yohimbine improved colonic blood flow in endotoxemic horses (8); however, its effect on small intestinal blood flow during endotoxemia has not been described for the horse. Yohimbine was not effective in blocking the clinical signs of endotoxemia, a not-unexpected finding, as clinical signs appear to be mediated, for the most part, by products of arachidonic acid metabolism (3,4). This finding is consistent with the results of a previous study in endotoxemic cows (9), where yohimbine (0.125 mg/kg, IV) had no effect on heart and respiratory rates.

$Alpha_2$ receptors are involved in the regulation of gastric tone. Stimulation of α receptors in gastric smooth muscle causes relaxation by a direct effect on postjunctional α_1 receptors, and an indirect action on α_2 adrenergic receptors located on the nerve terminals of cholinergic neurons (10). Activation of presynaptic α_2 receptors modulates acetylcholine output in the enteric nervous system (11). In vitro studies indicate that α_2 receptors are responsible for the presynaptic inhibition of neuronal fast and slow excitatory postsynaptic potentials (11). These actions would be expected to decrease gastric tone. In a traumatic ileus model in the horse, yohimbine (0.15 mg/kg, IV) was somewhat effective in restoring electrical and mechanical activity of the stomach and proximal jejunum (7). Endotoxin-induced reticulorumen stasis in cattle was partially restored by yohimbine (9). In that study, yohimbine did not block the clinical signs of endotoxemia, namely, altered body temperature, heart rate, and respiration rate. In rats, yohimbine reduced the severity of postoperative ileus (12).

The effects of endotoxin on the gastrointestinal tract are mediated by a multitude of factors. Endotoxemia causes an increase in sympathetic nerve activity (13) and plasma concentrations of catecholamines (14). Alpha-2 adrenergic receptor activity may also indirectly inhibit gastrointestinal motility, through an effect on arachidonic acid metabolism (15). Prostaglandins, products of arachidonic acid metabolism, are known to have modulatory effects on gastrointestinal motility, and are, in part, responsible for endotoxin-induced ileus (2). Pretreatment of horses with phenylbutazone, a cyclooxygenase inhibitor, prevented an endotoxin-induced delay in gastric emptying (3).

The dose of yohimbine used in this study, 0.25 mg/kg, IV, over 30 min, was higher than previously reported doses used in horses (8,16). Alpha-2 antagonists such as yohimbine have the potential to cause excitement. Yohimbine (0.12 mg/kg, IV), when given over an average time period of less than 10 min, produced mild excitation, muscle tremors and agitation in 2 of 4 horses (16). Although no excitement was observed in this study, the authors recommend that caution be exercised when administering yohimbine, and advise that the drug be infused over a minimum period of 60 min.

The use of acetaminophen as a liquid marker for determining the rate of gastric emptying in horses has been reported previously (5,6). The method has been used to study gastric emptying in dogs (17), rats (18), and humans (19). Although rapidly absorbed from the small intestine, acetaminophen is poorly absorbed from the stomach, and, therefore, can be used as an indirect indication of the rate of gastric emptying. A recent study in horses indicated that T_{max} was significantly correlated with the half-time of liquid phase emptying (T_{50}) using scintigraphy (20). The shortcomings of the acetaminophen method have been discussed previously (3,6), and factors other than gastric emptying could have influenced acetaminophen absorption. These factors include endotoxin-induced changes in intestinal blood flow (8) and permeability (21); however, endotoxin did not affect saline absorption in the rat duodenum or ileum (22). Endotoxin had no effect on intestinal transit in rats, but did cause a 7-fold delay in gastric emptying (21). Endotoxin could affect the elimination of acetaminophen by altering hepatic metabolism and splanchnic blood flow (23); however, these factors are not likely to influence T_{max}.

In conclusion, yohimbine was efficacious in blocking the delay in gastric emptying caused by endotoxin. Because yohimbine was not effective in blocking the clinical signs of endotoxemia, adjunctive treatment, such as administration of NSAIDs, may be indicated when treating endotoxin-induced ileus.

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