The effect of a pectin-lecithin complex on prevention of gastric mucosal lesions induced by feed deprivation in ponies

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Keywords: horse; pectin-lecithin complex; gastric lesions; feed deprivation; gastric squamous mucosa; ponies

Summary

This study examined whether a product containing a pectin-lecithin complex (Pronutrin) could prevent gastric lesions induced in the equine gastric squamous epithelial mucosa using a protocol of intermittent feed deprivation that resulted in prolonged increased gastric acidity (Murray and Eichorn 1996). Eight ponies were used and served as their own controls in 2 trials in which there were 72 h cumulative deprivation (alternating 24 h with no feed, then 24 h free choice hay), with a 4-week interval between trials. Ponies were assigned randomly to receive either 250 g Pronutrin plus 200 g pelleted feed, or 450 g pelleted feed only. Ponies were conditioned to each treatment for 7 days and received Pronutrin and pellets orally pellets once daily during the feed deprivation protocol. Gastroscopy was performed at the beginning and conclusion of the feed deprivation protocol. The endoscopist (M.J.M.) was blinded as to treatments, and lesion severity was scored on a scale of 0–5. Gastroscopy revealed normal-appearing gastric mucosa at the beginning of feed deprivation, with the exception of 2 ponies which had focal squamous mucosal erosion and 1 pony with focal glandularmucosal erosion. After 72 h cumulative feed deprivation, each pony, except 1 pony in one of the trials, developed erosions or ulcers in the gastric squamous mucosa. There was no difference (P = 0.6) in the presence or severity of gastric lesions between treatments. Lesions did not develop in the gastric glandular mucosa as a result of the intermittent feed deprivation with either treatment. In this study, the pectin-lecithin complex in Pronutrin failed to prevent lesions in the gastric squamous mucosa induced by intermittent feed deprivation.

Introduction

Gastric ulceration affects a large number of foals and horses, and the risk of ulceration appears to be related to training intensity, housing and feeding management (Murray and Eichorn 1996; Murray et al. 1996; Murray 1999; Vatistas et al. 1999a). Most lesions develop in the stratified squamous mucosal epithelium of the stomach (Murray et al. 1989; Vatistas et al. 1999b), and the predominant factor in the development of these lesions appears to be exposure of the squamous epithelium to hydrochloric acid (Murray and Eichorn 1996). Gastric ulcers can be induced consistently by exposure to prolonged periods of increased acidity, using a protocol of intermittent feed deprivation (Murray and Eichorn 1996; Murray 1994). In mature horses, lesions in the gastric glandular mucosa tend to predominate in the antrum (Murray et al. 2001) and, because of elaborate mechanisms of mucosal protection (Højgaard et al. 1996), excessive acidity is probably not the predominant factor in the development of lesions in that area. Suppressing gastric acidity using histamine type-2 receptor antagonists (H₂ antagonists) or the proton pump inhibitor omeprazole has been shown to be effective in treating (Furr and Murray 1989; Andrews et al. 1999) and preventing (Murray and Eichorn 1996; Andrews et al. 1999) lesions in the gastric squamous epithelial mucosa. While effective, these treatments have disadvantages that include required frequency of administration, expense and issues related to the use of medication in performance horses.

Methods to prevent or treat gastric ulcers effectively without requiring continued use of pharmaceutical agents would be desirable. In one report, administration of a natural food product containing a pectin-lecithin complex (Pronutrin) was reported to be associated with improved healing of gastric ulcers compared to untreated horses (Venner et al. 1999). Pronutrin consists of natural food sources of lecithin and pectins. Lecithin and related phospholipids have been proposed to be important in gastric mucosal protection, specifically by contributing to the hydrophobic barrier of the mucus layer overlying the gastric glandular mucosa (Lichtenberger 1996). Pectin-containing materials have been postulated to have similar properties (Dunji et al. 1993) and have been used to reduce exposure of oesophageal mucosa to acidic gastric reflux (Havelund et al. 1997).

The objective of this study was to determine whether Pronutrin was effective in preventing lesion formation in the gastric squamous mucosa in ponies using a feed deprivation ulcer-induction model.

Materials and methods

Animals

Eight mature ponies, 3 mares and 5 geldings, age 5–12 years and weighing 123 to 222 kg, were used. Ponies were given physical examinations, vaccinated for tetanus, influenza, equine herpesvirus 1 and 4, encephalitis and rabies, and administered

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TABLE 1: Gastric lesion scores after 72 h of cumulative feed deprivation for 2 replicates, Pronutrin (fed 250 g Pronutrin and 200 g pelleted feed) and No Pronutrin (450 g pelleted feed only). For more details, see the text. Ulcer scores used here are the highest scores for each of the regions of the squamous and glandular mucosal linings.

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<th>Pronutrin Glandular</th>
<th>No Pronutrin Squamous</th>
<th>No Pronutrin Glandular</th>
<th>Ulcer score difference (Pronutrin - No Pronutrin) Squamous</th>
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iwermectin at 200 µg/kg bwt. Ponies were housed in 20 x 40 m pens with run-in sheds, 4 ponies per pen, for 3 weeks before the study began. Ponies were fed timothy grass hay free choice, and were individually fed a pelleted feed (Bonanza 12P)\(^2\), increasing gradually from 250 g/day to a maintenance level of 450 g/day. Ponies were weighed using a calibrated electronic scale when purchased and at the beginning of the first and second trials.

Treatment protocol

The experiment was conducted in a 2-period, 2-treatment crossover design with 2 replications of each treatment within each treatment. Ponies served as their own controls, and there were 4 treated and 4 untreated ponies in each of 2 trials, with a 4 week interval between trials. This interval was chosen to allow any previously induced ulcers to heal completely. Blood was taken for haematology and serum chemistry profile at the beginning of the first trial. Blood was taken for packed cell volume (PCV) and total plasma protein (TPP) at the beginning and completion of each trial.

Pronutrin was supplied in a formulation of small pellets. Treated ponies received, once daily, 250 g Pronutrin with 200 g feed pellets, and untreated ponies received 450 g feed pellets. Ponies received Pronutrin plus feed pellets or just feed pellets for 7 days prior to beginning the ulcer induction protocol, to acclimate to the treatment and to allow time for the Pronutrin to have an effect on the gastric mucosa.

Ulcer-induction protocol

Ponies were brought into box stalls, 2 ponies per stall and 4 ponies at a time. Of the 4 ponies, 2 were in the Pronutrin-treated group and 2 were in the untreated group. Upon bringing them into stalls, the ponies were first fed timothy grass hay free choice for 12 to 24 h and then were muzzled to prevent eating, but not drinking from a water bucket, for 24 h. Ponies were alternately deprived of feed for 24 h, then had timothy grass hay available ad libitum for 24 h until feed was deprived cumulatively for 72 h. The protocol was approved by the Virginia Tech Animal Care Committee.

During the feed deprivation protocol, ponies received either 250 g Pronutrin with 200 g pelleted feed or 450 g pelleted feed daily. Feeding this amount of pellets during the feed deprivation protocol was not expected to have an impact on induction of gastric lesions.

Gastroscopy

Prior to gastroscopy, each pony was sedated with 0.02 mg/kg bwt acepromazine i.v. and then 0.5 mg/kg bwt xylazine i.v. Gastroscopy was performed 9–12 h after feed deprivation began on Day 1, to ensure that the mucosal linings of the stomachs were normal. Thereafter, during the feed deprivation, protocol gastroscopy was performed after 72 h of cumulative feed deprivation.

Gastroscopy was performed with a 2 m long video endoscope\(^3\). The endoscopist (M.J.M.) was blinded as to treatment received by the ponies during the trial. Images were digitised and captured during the examination using a PC computer with a Targa board. Images were taken of the gastric squamous epithelium from the right side of the stomach along the margo plicatus (MPRT), the dorsal fundus (FUND), the greater curvature along the margo plicatus (MPGC), the lesser curvature along the margo plicatus (LC), and the glandular mucosa of the body (GL) and the antrum/ pylorus (ANT).

Statistical analysis

Lesions were scored from digitised images on a scale of 0–5 using the following criteria. Grade 0: the epithelium is intact and there is no appearance of hyperaemia (reddening) or hyperkeratosis (yellow appearance to the squamous mucosa); Grade 1: the mucosa is intact, but there are areas of reddening or hyperkeratosis (squamous); Grade 2: small single or multifocal lesions which appear superficial; Grade 3: extensive lesions which appear superficial; Grade 4: large single or multifocal lesions, some may appear deep; Grade 5: extensive lesions with areas of apparent deep ulceration.

Pairwise comparison (Pronutrin plus pelleted feed vs. pelleted feed) of lesion scores after 72 h cumulative feed deprivation were made using the Wilcoxon signed rank test. During the trials it was noted that the packed cell volume (PCV) of some ponies appeared low and total plasma solids appeared increased. The GLM procedure of the SAS System (ver. 7.01)\(^4\) was used to perform ANOVA to test for treatment effect on PCV and total solids.

Results

Initially, ponies did not readily eat the Pronutrin pellets, but after 2 or 3 days they accepted the Pronutrin and ate all that was
provided. All ponies gained weight during the study.

Daily assessment of temperature, heart rate and other clinical observations performed while the ponies were in stalls were normal. Results of haematology and serum chemistry were normal, except for anaemia in some ponies (PCV range 23–40%).

There was no treatment effect (Pronutrin plus pellets compared to pellets) on PCV or total solids on Days 1 or 5 of the trials (P = 0.5). Faecal floatations of pooled faecal specimens from ponies in their respective pens were negative for parasite ova, and the ponies were administered another 200 µg/kg bwt dose of ivermectin between the first and second feed deprivation trials.

Gastroscopy revealed normal gastric mucosal integrity at the beginning of feed deprivation, with the exception of 2 ponies which had focal squamous mucosal erosion and one pony with focal glandular mucosal erosion (Table 1). After 72 h cumulative feed deprivation, each pony except one developed lesions (score of 2 or greater) in the gastric squamous mucosa. There was no difference (P = 0.6) in development of gastric lesions or lesion severity based on the administration of Pronutrin. Feed deprivation was not associated with development of lesions in the glandular mucosa of the gastric body or antrum/pylorus, which is similar to results of previous feed deprivation studies that have been performed (Murray and Eichorn 1996; Murray 1994).

Discussion

The pectin-lecithin complex in Pronutrin consists of an amphiphilic phospholipid (lecithin) and a hydrophilic, gel-forming carbohydrate polymer (pectin), which are bound into a complex (Apolectol) that is proposed to augment the hydrophobic properties of gastric mucus that repel aqueous hydrochloric acid. The hydrophobic mucus barrier that overlies the gastric glandular mucosa is considered to be a key component of the inherent resistance of that mucosa to damage from acidic peptic secretions (Lichtenberger 1995). However, the equine stomach is lined by a stratified squamous mucosa dorsally and a glandular mucosa ventrally. The equine gastric squamous mucosa has no mucus layer to prevent contact with acidic peptic secretions (Murray and Mahaffey 1993) and this mucosa appears to be highly susceptible to peptic injury (Murray and Eichorn 1996).

The equine stomach secretes hydrochloric acid continuously (Campbell-Thompson and Merritt 1990), and feed deprivation in horses is associated with highly acidic conditions within the stomach (Murray and Schusser 1993). Using the feed deprivation protocol, we have shown previously that the acid-suppressive agent ranitidine prevented or minimised ulcer development in the gastric squamous mucosa (Murray and Eichorn 1996). The failure of Pronutrin to prevent lesion development implies that it neither affects gastric acidity nor provides protection to the gastric squamous epithelial mucosa.

In a recent report (Venner et al. 1999), Pronutrin administration appeared to be associated with improved healing of gastric squamous mucosal lesions. However, in that study horses were not allocated randomly to treatment groups, the endoscopist was not blinded as to treatment and the trial lasted only 14 days. That may be an insufficient time frame in which to evaluate the effect of a compound that may facilitate gastric ulcer healing, because spontaneous healing of gastric squamous mucosal lesions can occur in 14 days (Murray et al. 1997), clouding any comparison of ulcer healing in treated and untreated animals.

Alternatively, it is possible that Pronutrin may enhance ulcer healing while having no effect on gastric acidity or mucosal protection from peptic injury. The pectin-lecithin complex in Pronutrin may facilitate ulcer healing indirectly by binding to ulcerated squamous mucosa, or it may have a direct effect on ulcer healing. Exogenous substances have augmented ulcer healing in laboratory animals. Angiogenesis and ulcer healing have been enhanced by administration of fibroblast growth factor in rats (Szabo et al. 1994), and aluminum-containing agents such as sucralfate and antacids increased mucosal blood flow by promoting nitric oxide and prostaglandin synthesis (Konturek et al. 1995; Lambrecht et al. 1993). These latter effects, however, have been examined in gastric glandular or duodenal mucosa; similar effects in alimentary squamous mucosa have not been reported and, other than the report by Venner et al. (1999), there are no reports to date that support a role for the ingredients in Pronutrin in healing of equine gastric squamous mucosal ulcers.

Further studies are necessary to determine whether Pronutrin may augment healing of equine gastric ulcers. Based on the results of this study, prophylaxis of gastric squamous mucosal lesions in horses does not appear to be an attribute of Pronutrin at the amount administered to our ponies.

Acknowledgement

This study was supported by a grant from Boehringer Ingelheim, Denmark.

Manufacturers’ addresses

1Boehringer-Ingelheim, Copenhagen, Denmark.
2Cooperative Milling, Gettysburg, Pennsylvania, USA.
3Welch Allyn, Skaneateles Fall, New York, USA.
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Received for publication: 5.9.00

Accepted: 12.4.01