The Protective Effects of Sucralfate and Ranitidine in Foals Experimentally Intoxicated with Phenylbutazone

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ABSTRACT

The effects of sucralfate and ranitidine on the gastrointestinal manifestations of phenylbutazone (PBZ) toxicity in horse foals were determined by complete blood count, serum chemistry profile, and gross and histological necropsy examinations. Twenty-eight, three to four month old Belgian-cross foals were randomly assigned to one of four groups. Phenylbutazone was administered at a dosage of 10 mg/kg of bodyweight (BW) per day, intravenously (IV), in equally divided doses to three of the groups. In addition to PBZ, ranitidine was administered at 2 mg/kg BW, IV, twice daily, to one group of seven foals (PBZ/ranitidine group), and sucralfate was administered at 4 g, orally, twice daily to another group of seven foals (PBZ/sucralfate group). A fourth group received normal saline IV and corn syrup orally, twice daily, as placebos (control group). Treatments were administered for ten days.

Clinical signs included oral ulceration (in all PBZ-treated foals) and diarrhea (5/7 and 2/7 foals from the PBZ and PBZ/ranitidine groups, respectively). A reduction in total protein and albumin was greatest in the PBZ group and least in the PBZ/ranitidine and PBZ/sucralfate groups when compared to the control group. The PBZ group lost weight during the treatment period.

At necropsy, the PBZ group had the greatest area of oral ulceration compared to the other treatment groups. All foals treated with PBZ had gastric ulcers; however, the PBZ group had the most severe gastric epithelial necrosis compared to the other three treatment groups.

Duodenal villous atrophy, epithelial necrosis and mucosal inflammation, and a reduction in epithelial mitotic figures were seen in all PBZ-treated foals. Large intestinal ulceration and colonic epithelial necrosis and mucosal inflammation were greater in the PBZ group compared to the other treatment groups. It was concluded that sucralfate and ranitidine provided partial protection against the clinical, clinicopathological and pathological manifestations of phenylbutazone toxicity.

RÉSUMÉ

Cette expérience visait à déterminer les effets de la ranitidine et du sucralfate sur les manifestations gastro-intestinales de la toxicité de la phénylbutazone, chez des poulains, par la numération globulaire, le profil de la chimie sérique, la recherche de lésions macroscopiques, lors de la nécropsie, et l'histopathologie. Les auteurs utilisèrent à cette fin quatre groupes de sept poulains Belges croisés, âgés de trois à quatre mois. Les sujets des groupes A, B et C reçurent, pendant dix jours, une injection intraveineuse quotidienne de phénylbutazone, à raison de 100 mg/kg. Les sujets du groupe B reçurent, en plus de la quantité précitée de phénylbutazone, deux injections intraveineuses quotidiennes de ranitidine, à raison de 2 mg/kg par injection. Les sujets du groupe C reçurent, en plus de la quantité précitée de phénylbutazone, 4 g de sucralfate, par la voie buccale, deux fois par jour. Les sujets du groupe D, les témoins, ne reçurent que de l'eau physiologique et du sirop de maïs, par la voie buccale, deux fois par jour. Tous les poulains des groupes A, B et C développèrent des ulcères buccaux, alors que cinq des sept du groupe A et deux des sept du groupe B manifestèrent aussi de la diarrhée. La baisse des protéines totales et de l'albumine s'avéra maximale, chez les poulains du groupe A, et minimale, chez ceux des groupes B et C, par comparaison avec les témoins. Les poulains du groupe A accusèrent aussi une perte de poids, au cours des dix jours que dura l'expérience.

La nécropsie révéla une ulcération buccale beaucoup plus sévère, chez les poulains du groupe A que chez ceux des groupes B et C. Tous les sujets des groupes A, B et C présentaient des ulcères gastriques; ceux du groupe A affichaient cependant la nécrose épithéliale gastrique la plus sévère.

Le duodénum de tous les poulains des groupes A, B et C présentait de l'atrophie des villosités, de la nécrose épithéliale, une inflammation de la muqueuse et une diminution des mitoses épithéliales.

L'ulcération, la nécrose épithéliale et l'inflammation du côlon se révélèrent plus marquées chez les poulains

du groupe A que chez ceux des groupes B et C.

Il semble par conséquent que la ranitidine et le sucralfate protégèrent partiellement le tube digestif contre les manifestations cliniques, clinicopathologiques et pathologiques de la toxicité de la phénylbutazone.

INTRODUCTION

Gastroduodenal ulceration is recognized as an important disease in foals one week to six months of age (1-4). Active ulcers are often manifest by abdominal pain, excessive salivation and bruxism (1). The clustering of affected foals on certain farms has suggested an infectious etiology but attempts to identify a causative microorganism have been unsuccessful (2,5). Parasitic damage (6), congenital anomalies (7) and stress (3) have all been implicated in the pathogenesis of these ulcers. A recent necropsy study which reported a high prevalence of gastric and duodenal ulcers in foals treated with nonsteroidal antiinflammatory drugs (NSAIDs) suggested that these agents may be one cause of gastrointestinal ulceration in foals (4).

Phenylbutazone (PBZ) is the most widely used NSAID in equine medicine (8,9), despite its reported toxicity (10-14). Ulceration of the alimentary tract is the major manifestation of PBZ toxicity and gastric ulceration has been reported in foals given 10 mg PBZ/kg/day for 10-42 days (8).

Drugs, such as the histamine H_2-receptor antagonists, and sucralfate, an aluminum salt of sucrose octasulphate, which have proven effective in the treatment of human gastroduodenal ulceration have been used empirically in foals (1). Pharmacologically, sucralfate polymerizes in an acid environment, preferentially adhering to injured tissue and forming a protective barrier (15,16). It has also been shown to significantly reduce gastric injury associated with the use of NSAIDs in humans (17,18).

The histamine H_2-receptor antagonists, cimetidine and ranitidine, block the effect of histamine on parietal cells and depress both basal and stimulated gastric acid secretion (19,20). Ranitidine also has been shown to have a protective action on gastric mucosa exposed to NSAIDs, probably through decreased acid secretion (21). Furthermore, in horses it has been shown to depress gastric acid secretion (22) but its potential as an antiulcerogenic compound in the horse has not been investigated.

The ubiquitous use of NSAIDs in foals is cause for concern because of the possibility of gastroduodenal ulceration. Therefore, there is a need to identify drugs which may aid in the prevention of NSAID-induced ulceration and evaluate their potential efficacy. The objective of this study was the evaluation of the effect of sucralfate and ranitidine on the clinical and pathological manifestations of phenylbutazone toxicity.

MATERIALS AND METHODS

FOALS

Experiments followed guidelines equivalent to those in "Guide to the Care and Use of Experimental Animals", Volumes 1 and 2, Canadian Council on Animal Care. Twenty-eight three to four month old Belgian cross foals (15 female and 13 male) were maintained outdoors in a yard measuring 75 x 25 meters. The foals were weaned and treated with a broad spectrum anthelmintic (Ivermectin, "Eqvalan", MSD AGVET, Canada) three weeks and ten days prior to the commencement of the study, respectively. Alfalfa hay, salt blocks containing trace minerals and water were all available ad libitum throughout the study period.

TREATMENTS

Each foal was randomly assigned to one of four groups. Treatments were given according to the schedule given in Table I. The PBZ dosage of 10 mg/kg/day for ten days was chosen because this dosage has been reported to cause gastric ulceration in horses (10,13). The dosages of ranitidine and sucralfate were extrapolated from human literature (16,19,20) as limited equine data were available. Treatment was continued for ten days. Only one investigator (R.J.G.) was aware of treatments given to each foal.

TABLE I. Treatment Regimens for Experimental Foals

Group	Dose	Route
PBZ[a]	5 mg/kg bwt [+b]	IV
PBZ and	5 mg/kg bwt	IV
rantitidine[c]	2 mg/kg bwt	IV
PBZ and	5 mg/kg bwt	IV
sucralfate[d]	4 g	PO
Control (normal saline)	5 mL	IV

[+]All treatments were administered at 12 h intervals
[a]PBZ = Phenylbutazone (Butazone, rogar/STB Inc., London, Ontario)
[b]bwt =bodyweight
[c]Zantac, Glaxo Laboratories Inc., Toronto, Ontario
[d]Sulcrate, Nordic Laboratories, Montreal, Quebec; dissolved in water and mixed with corn syrup. Corn syrup was also administered to the other three treatment groups

PHYSICAL EXAMINATION

All foals were examined twice daily. In addition, the foals were observed in their yard for one hour each morning. Body temperature, fecal consistency and appetite were monitored and recorded daily. The foals were weighed on days 0, 5 and 10, and average daily weight gain was calculated. Following the weighing on day 5, the dosage of each drug was adjusted if necessary.

CLINICAL PATHOLOGY

Blood samples were collected into evacuated tubes (Monoject Blood Collection Tubes, Monoject, St. Louis, Missouri) on days 0, 5 and 10, for a complete blood count (CBC) and determination of serum total protein, albumin, sodium, potassium, chloride, urea, creatinine, alkaline phosphatase, aspartate aminotransferase, gamma-glutamyl transferase, calcium and phosphorus. Complete blood counts were determined on a Coulter Counter (Coulter + IV, Coulter Electronics Ltd., Hialeah, Florida), while serum chemistry analysis was performed on an autoanalyzer (Dacos, Coulter Electronics Ltd., Hialeah, Florida).

Serum pepsinogen concentrations were determined on samples collected on days 0, 5 and 10 by the method described by Edwards et al (23). Pepsinogen activity was expressed as international units (IU) where one unit equals 1 μmol tyrosine released per L of serum per min at 37°C.

TABLE II. Median Bodyweights and Average Daily Gain for Foals Treated with Phenylbutazone (PBZ), PBZ and Ranitidine, PBZ and Sucralfate, or Normal Saline for Ten Days

Day	PBZ	PBZ and Ranitidine	PBZ and Sucralfate	Control
Body weight (kg)				
0	181 ± 23[a]	181 ± 32	200 ± 15	204 ± 21
5	182 ± 19	184 ± 36	204 ± 20	199 ± 25
10	177 ± 20	180 ± 21	206 ± 15	199 ± 22
Average daily gain	-0.7 ± 1.0[b]	0.9 ± 2.0	0.6 ± 0.7	0.8 ± 1.0

[a]Values are medians with 25th percentiles
[b]Significantly different from other three groups (p < 0.05)

PATHOLOGICAL EXAMINATION

All foals were necropsied on treatment day 10 following euthanasia with pentobarbital (Euthanyl Forte, M.T.C. Pharmaceuticals, Mississauga, Ontario). The pathologist who conducted the necropsy and the histopathological examination of tissues was not aware of the treatment groups. Gastrointestinal tract (whole), stomach, small intestine, large intestine, kidney and liver weights were recorded. Each oral ulcer was measured with a standard metric rule, and the area of oral cavity ulceration was calculated. The entire length of the gastrointestinal tract was opened and examined; numbers of ulcers in the esophagus, nonglandular (NGS) and glandular stomach (GS) were recorded. Ulceration in the small and large intestine was graded on the following scale: 0, no ulcers; 1, mild ulceration (< 5 ulcers); 2, moderate ulceration (> 5 ulcers, but no mucosal petechiation); 3, severe ulceration (> 5 ulcers and mucosal petechiation). The mucus covering on the GS was assessed using the following scale: 0, normal (mucus layer 1-2 mm deep); 1, reduced (mucus layer < 1 mm deep); 2, absent (no mucus layer visible).

Standard tissue sections of NGS and GS from the mid-point of the greater curvature of the stomach, duodenum 10 cm distal to the pylorus, cecum 10 cm from the apex, and ascending colon 10 cm from the diaphragmatic flexure, were collected and fixed in Bouin's solution for histological examination. Sections of liver and kidney were also collected and fixed in 10% buffered formalin.

HISTOPATHOLOGICAL EXAMINATION

For each section of NGS and GS, duodenum and colon, the width of the mucosa, submucosa and muscularis layers was measured from a low power microscopic image projected onto a television monitor. The degree of epithelial necrosis, villous atrophy and mucosal inflammation was graded on the following scale: 0, normal; 1, mild (epithelial cell atrophy, normal duodenal villi, and a mild inflammatory infiltrate into the lamina propria); 2, moderate (areas of epithelial necrosis, stunting of duodenal villi, and a moderate inflammatory infiltrate into the lamina propria); 3, severe (total loss of epithelium, complete destruction of duodenal villi, and a marked inflammatory infiltrate

into the lamina propria). The duodenal and colonic epithelial mitotic indices were recorded by taking the median number of mitoses seen in four high-power fields of the epithelium. To determine the area of the gut associated lymphoid tissue (GALT) of the colon, the average diameter of three follicles was recorded. The degree of depletion of mature lymphoid forms and necrosis of the GALT was graded using the following scale: 0, normal; 1, mild (some immature lymphoid cells present, no necrosis); 2, moderate (few mature lymphoid cells present, some cells necrotic); 3, severe (no mature lymphoid cells present, most cells necrotic).

STATISTICAL ANALYSIS

The data were evaluated using a computer based statistical program (SAS Institute Inc., Raleigh, North Carolina). For each variable, normal probability plots were used to check for normal distribution of the data. The clinical pathology data, with the exception of gamma-glutamyl transferase and immature neutrophil counts, were normally distributed and analyzed by a two-way analysis of variance with repeated measures on the factor of time. A Tukey's test was applied to variables showing significant between group differences. The nonparametric clinical pathology and pathology data were ranked, and a Kruskal-Wallis one-way analysis of variance applied. The Bonferroni multiple comparison test was used to locate significant differences among medians determined by the initial analysis of variance (24). Statistically significant differences were assumed to exist when the probability of making a type I error was < 0.05.

RESULTS

CLINICAL FINDINGS

The main clinical findings were oral ulceration and diarrhea with associated signs of excess salivation and depression in all three groups that received PBZ. The control group appeared clinically normal except for one foal in which icteric mucous membranes and evidence of photosensitization was noted on treatment day (TD) 8. The average daily weight gain of the PBZ group foals was significantly lower than the other three groups (Table II). All foals maintained normal appetite during the study period. Five foals from the PBZ group developed diarrhea between TD 3 and

TABLE III. Mean Serum Values for Calcium, Total Protein and Albumin from Foals Treated with Phenylbutazone (PBZ), PBZ and Ranitidine, PBZ and Sucralfate, or Normal Saline for Ten Days

Day	PBZ	PBZ and Ranitidine	PBZ and Sucralfate	Control
Calcium (mmol/L)				
0	3.17 ± 0.1[a+]	3.18 ± 0.2[a]	3.15 ± 0.06[a]	2.96 ± 0.5[a]
5	2.72 ± 0.08[a]	3.01 ± 0.24[b]	3.10 ± 0.18[b]	3.11 ± 0.18[b]
10	2.70 ± 0.28[a]	2.94 ± 0.35[b]	2.98 ± 0.16[b]	3.10 ± 0.07[b]
Total Protein (g/L)				
0	64.7 ± 6.47[a]	64 ± 3.2[a]	63.9 ± 4.3[a]	63.3 ± 2.8[a]
5	50.1 ± 5.3[a]	59.3 ± 5.3[b]	56.7 ± 3.1[b]	64.4 ± 4.7[c]
10	49.1 ± 4.3[a]	58.6 ± 9.9[b]	54 ± 8.75[b]	64.7 ± 6.5[c]
Albumin (g/L)				
0	28.7 ± 3.1[a]	28.9 ± 2.6[a]	31 ± 1.6[a]	29.7 ± 2.7[a]
5	21.7 ± 1.1[a]	25.3 ± 2.9[b]	25.9 ± 2.6[b]	29.1 ± 2.6[c]
10	15.7 ± 2.5[a]	22.6 ± 5.2[b]	23.1 ± 4.2[b]	28.7 ± 3.1[c]

+Values are means with standard deviations
[abc]Values with different letter superscripts are significantly different (p < 0.05)

10. The feces were initially soft and fluid, but in two foals became frequent and watery. Two PBZ/ranitidine group foals developed soft, fluid feces on TD 9. Diarrhea was not noted in any foal in the PBZ/sucralfate or control groups.

In the PBZ group oral ulcers first were noted in all foals between TD 2 and 5. Ulcers were circular to oblong, punctate, and particularly prominent at the lip commissures, the mucocutaneous junctions, and on the mucosa of the gingiva and tongue. Three foals from the PBZ group were severely affected, and excess salivation and depression accompanied the oral ulceration. Two foals developed ulceration at the ventral portion of both nostrils. All foals in the PBZ/ranitidine-treated group developed oral ulcers; these were first noted on TD 3 in two foals, and between TD 6 and 8 in the other foals. Two foals with severe oral ulceration had accompanying signs of excess salivation and depression. Five foals in the PBZ/sucralfate group were first noted to have oral ulcers between TD 4 and 8. None of these foals exhibited excess salivation or depression.

CLINICAL PATHOLOGY

No significant differences were noted among the groups in any of the serum parameters determined except for the concentrations of total protein, albumin and calcium (Table III). Significant reductions in both total protein and albumin concentrations were observed in the PBZ, PBZ/ranitidine and PBZ/sucralfate groups in comparison to the control group. This reduction was significantly greater in the PBZ group compared to the other three groups, and accompanied by a significant fall in serum calcium. Two foals from the PBZ group, and one from each of the PBZ/ranitidine and control groups had elevated values of aspartate aminotransferase and gamma-glutamyl transferase on TD 0.

The PBZ and PBZ/ranitidine groups showed an increase in immature neutrophil counts on days 5 and 10 (Table IV).

The serum pepsinogen concentrations on days 5 and 10 were not different from baseline values, and no significant differences were seen among treatment groups (Table V).

TABLE IV. Mean and Median Hematology Values from Foals Treated with Phenylbutazone (PBZ), PBZ and Rantidine, PBZ and Sucralfate, or Normal Saline for Ten Days

Day	PBZ	PBZ and Ranitidine	PBZ and Sucralfate	Control
Packed Cell Volume (L/L)				
0	$0.33 \pm 0.03^+$	0.32 ± 0.05	0.33 ± 0.02	0.31 ± 0.03
5	0.31 ± 0.02	0.32 ± 0.04	0.32 ± 0.01	0.31 ± 0.03
10	0.33 ± 0.05	0.31 ± 0.04	0.31 ± 0.02	0.33 ± 0.03
Fibrinogen (g/L)				
0	$4.7 \pm 1.3^+$	5 ± 0.8	6 ± 2.4	4.7 ± 1.9
5	5.6 ± 1.7	5.7 ± 1.5	4.8 ± 1.6	4.9 ± 1.8
10	8.4 ± 2.6	5.7 ± 1.6	5 ± 2.1	4.7 ± 1.2
Total Leukocyte Count (cells/L x 10⁹)				
0	$9.3 \pm 2.2^+$	10.9 ± 2.6	9.5 ± 2.4	9.8 ± 2.6
5	7.4 ± 1.7	9.7 ± 1.9	7.9 ± 1.7	9.2 ± 2.2
10	15.4 ± 4.6	15.1 ± 4.6	11 ± 3.9	9.3 ± 2.2
Mature Neutrophil Count (cells/L x 10⁹)				
0	$3.9 \pm 1.7^+$	5.4 ± 1.9	4 ± 1.4	4.2 ± 1.9
5	1.0 ± 0.8	2.8 ± 2.1	2.5 ± 1.3	3.7 ± 2.2
10	5.3 ± 1.9	7.9 ± 4.1	4.8 ± 3.6	3.9 ± 2.2
Immature Neutrophil Count (cells/L x 10⁹)				
0	$0 \pm 0^{++}$	0.07 ± 0.04	0 ± 0	0 ± 0
5	0.28 ± 0.19^a	0.18 ± 0.15^a	0 ± 0	0 ± 0
10	0.44 ± 0.39^a	0.53 ± 0.48^a	0 ± 0	0 ± 0

+Values are means with standard deviations
++Values are medians with 25th percentiles
aSignificantly different ($p < 0.05$) from PBZ/sucralfate and control groups

PATHOLOGY

The pathological findings are presented in Table VI. The control group had no visible lesions except for the foal in which evidence of photosensitization was observed. All foals treated with PBZ had mild to severe ulceration of the oral cavity, particularly involving the lips, tongue, hard and soft palates, and labial and periodontal mucosa. Prominent separation of the periodontal mucosa from the molar teeth and a large variation in the size and shape of the ulcers was observed (Fig. 1). The area of oral ulceration was significantly greater in the PBZ group than the other treatment groups.

One foal from the PBZ group had marked submandibular and pharyngeal edema that was not clinically apparent. This foal also had approximately 2 L of clear, aseptic abdominal fluid. Two other foals from the PBZ group had approximately 2 L of yellow, turbid abdominal fluid; cytologically this fluid was characteristic of a suppurative exudate although no bacteria were cultured. Multiple small (< 1 cm) ulcers were seen in the proximal esophagus of two PBZ group foals, three PBZ/ranitidine group foals, and one PBZ/sucralfate group foal, but there were no significant differences between treatment groups.

All foals from the PBZ, PBZ/ranitidine and PBZ/sucralfate groups had ulcers in either the GS or the NGS, and there were significantly more ulcers in these groups when compared to the control group which had no gastric ulcers. The size of the ulcers was highly variable, ranging from 2 mm to 2 cm in diameter (Fig. 2). The larger ulcers were 1-2 mm in depth and

TABLE V. Mean Serum Pepsinogen Values (IU) from Foals Treated with Phenylbutazone (PBZ), PBZ and Ranitidine, PBZ and Sucralfate, or Normal Saline for Ten Days

Day	PBZ	PBZ and Ranitidine	PBZ and Sucralfate	Control
0	0.95 ± 0.16^a	0.61 ± 0.15	0.52 ± 0.24	0.56 ± 0.3
5	1.07 ± 0.18	0.88 ± 0.14	1.01 ± 0.15	1.04 ± 0.23
10	0.84 ± 0.11	0.81 ± 0.07	0.86 ± 0.10	0.84 ± 0.14

aValues are means with standard deviations
IU = international units

TABLE VI. Median Values of Area of Oral Ulceration and Numbers of Nonglandular (NGS) and Glandular (GS) Stomach Ulcers of Foals Treated with Phenylbutazone (PBZ), PBZ and Ranitidine, PBZ and Sucralfate, or Normal Saline for Ten Days

	PBZ	PBZ and Ranitidine	PBZ and Sucralfate	Control
Oral ulceration (cm²)	38.56 ± 29.3[a+]	5.86 ± 11.21[b]	6.16 ± 2.42[b]	1.5 ± 6.0[b]
Number of NGS ulcers	0 ± 4[a]	1 ± 4[a]	4 ± 5[a]	0 ± 0[b]
Number of GS ulcers	12 ± 13[a]	3 ± 7[a]	1 ± 9[a]	0 ± 0[b]

[+]Values are medians with 25th percentiles
[ab]Values with different letter superscripts are significantly different ($p < 0.05$)

were surrounded by hyperemia. The ulcers in the PBZ/sucralfate group appeared to be less active, and the mucosa surrounding the ulcers was considerably less hyperemic. Multiple linear fissuring of the GS mucosa was a prominent feature of five, two and two foals from the PBZ, PBZ/ranitidine and PBZ/sucralfate treatment groups respectively. The mucus covering of the GS mucosa in the PBZ and PBZ/ranitidine groups was significantly less than in the other two groups. Hyperkeratosis affecting 20 to 50% of the NGS was observed in five and three foals in the PBZ and PBZ/ranitidine groups respectively.

The small intestine of one foal in the PBZ group had five small (5 mm) circular ulcers in the anterior duodenum; one had severe ileal and jejunal edema, and another had an eroded hemorrhagic jejunoileal mucosa with enlarged Peyer's patches. One foal from the PBZ/sucralfate group had

multiple petechial hemorrhages on the duodenal, jejunal and ileal mucosae. However, no significant differences among the four treatment groups were observed.

Lesions of the large intestine were more frequently observed, and were particularly severe in the colon. All but one foal in the PBZ group had colonic lesions, characterized by multiple superficial ulceration and petechial hemorrhage affecting greater than 50% of the mucosa. Three of these foals had similar lesions in the cecum, and one foal had pronounced cecal and colonic edema. Similar lesions of mucosal ulceration and petechiation were seen in one foal from each of the PBZ/ranitidine and PBZ/sucralfate groups. Overall, the ulceration score for the PBZ group was significantly greater than for the other three groups.

The kidneys appeared normal except in three foals from each of the

PBZ, PBZ/ranitidine and PBZ/sucralfate groups which had areas of cortical pallor. In each of the PBZ and PBZ/ranitidine groups, two foals had embolic pneumonia. These foals also had thrombophlebitis of the jugular veins.

Liver lesions were seen in a limited number of foals in all treatment groups. Three foals from the PBZ group, two from the PBZ/ranitidine group, and one each from the PBZ/sucralfate and control groups were affected. The lesions varied from focal to diffuse areas of pallor with accentuation of the lobular pattern. One of the PBZ group foals had multiple 1 mm white foci at the periphery of the liver lobes. The liver of the control foal was markedly enlarged and had an accentuated lobular pattern.

No differences were seen concerning body score and relative organ weights among treatment groups.

HISTOPATHOLOGY

The histopathological findings are presented in Tables VII and VIII. Lesions of the gastric mucosa varied in severity, ranging from atrophy to erosion and ulceration. The PBZ group had a significantly greater score for gastric epithelial necrosis when compared to the other three groups. Five foals from the PBZ group had a moderate gastritis characterized by multifocal accumulations of plasma cells, lymphocytes and macrophages in the lamina propria and submucosal areas. In three of these foals the chief, parietal and mucus-producing cells could not be differentiated. In each of the PBZ/ranitidine and PBZ/sucralfate groups, one foal had a moderate gastritis with submucosal edema, and again it was not possible to differentiate the chief, parietal and mucus-producing cells. Gastric mucosa, submucosa and muscularis widths did not differ among treatment groups.

The PBZ, PBZ/ranitidine and PBZ/sucralfate groups all showed moderate to severe duodenal villous atrophy and duodenal epithelial necrosis and mucosal inflammation. There was a significant reduction in duodenal mucosal width and significantly higher scores for both epithelial necrosis and mucosal inflammation in these three groups compared to the
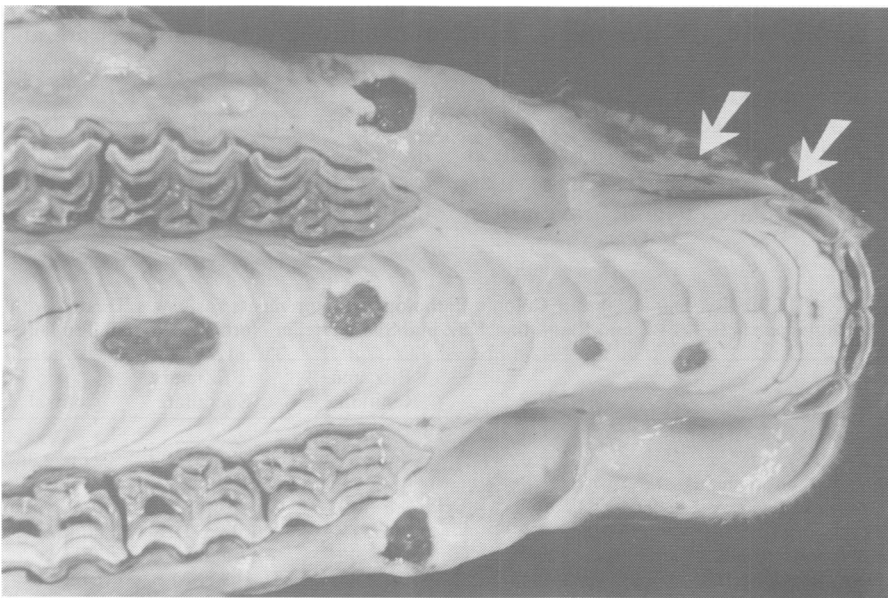

Fig. 1. Punctate ulceration of the lips (arrow), buccal mucosa and hard palate in a foal treated with phenylbutazone at 10 mg/kg/day for ten days.

control group. Three foals from each of the three treatment groups had total loss of villous structure with epithelial necrosis and, where epithelium remained, synechia formation. A moderate infiltration of lymphocytes and macrophages into the lamina propria was seen. In addition, there were reduced numbers of mitotic figures in the mucosal epithelium compared to the control group. No differences were seen in duodenal submucosal and muscularis measurements among treatment groups.

The scores for colonic epithelial necrosis and mucosal inflammation were significantly greater in the PBZ group compared to the other three groups. Prominent features were epithelial necrosis and ulceration, mononuclear cell infiltration into the lamina propria, and marked submucosal edema and fibrosis. By comparison, the PBZ/sucralfate and PBZ/ranitidine groups had only mild to moderate epithelial cell atrophy. The number of colonic epithelial mitotic figures was reduced particularly in the PBZ group, but also in the PBZ/ranitidine and PBZ/sucralfate groups, when compared to the control group. The PBZ group had smaller, depleted GALT in comparison to the other three groups. No group differences were seen for colonic mucosa and mucularis widths.

Few renal lesions were seen histologically; two foals from each of the PBZ, PBZ/ranitidine, and PBZ/sucralfate groups showed very mild proximal convoluted tubular degeneration. The liver lesions in the six foals with gross lesions consisted of a chronic, active cholangiohepatitis characterized by extensive bile duct hyperplasia, fibrosis and, in some centralobular areas, coagulative necrosis. The inflammatory reaction consisted of macrohages, lymphocytes, plasma cells and a few giant cells. Severe periportal fibrosis and bile duct hyperplasia were seen in the clinically affected foal from the control group.

DISCUSSION

The objective of this experiment was to create a model for gastrointestinal ulceration in foals so that the

Fig. 2. Stomach from a foal treated with phenylbutazone at 10 mg/kg/day for ten days showing deep, punctate glandular stomach ulcers with hemorrhage (arrow).

efficacy of the antiulcer drugs, ranitidine and sucralfate, could be determined. The model consisted of phenylbutazone administered to foals at a dosage of 10 mg/kg BW daily for ten consecutive days. Gastroduodenal ulcer disease in foals is associated with ulceration of the esophagus, nonglandular stomach and anterior duodenum (1,2,5). In this study oral, glandular and nonglandular stomach, cecal and colonic ulceration was seen. These findings are similar to those previously reported (10-14).

Gastrointestinal ulceration associated with PBZ administration is probably mediated via inhibition of cyclooxygenase with subsequent inhibition of prostaglandin synthesis (25). Prostaglandins (PG), particularly PGE_2, are integrally involved in

mucosal defense mechanisms, both by inhibition of gastric acid secretion, and by cytoprotection. Although no one mechanism fully explains cytoprotection, it is believed to involve an increase in gastroduodenal mucus and bicarbonate secretions, an increase in gastric mucosal blood flow, and stimulation of migration of mucosal epithelial cells toward the surface (26,27). The mucus secreted by the epithelial cells is composed of an adherent layer of gel glycoprotein and it protects the epithelium by slowing the diffusion of acid and pepsin, and by trapping bicarbonate to create an alkaline interface (28).

The net effect of NSAIDs is to increase mucosal susceptibility to the damaging effects of aggressive luminal factors, such as acid and pepsin.

TABLE VII. Median Duodenal and Colonic Pathology Measurements for Foals Treated with Phenylbutazone (PBZ), PBZ and Ranitidine, PBZ and Sucralfate, or Normal Saline for Ten Days

	PBZ	PBZ and Ranitidine	PBZ and Sucralfate	Control
Duodenal mucosal width (μ x 10^3)	1.45 ± 0.54[a+]	1.45 ± 0.54[a]	2.0 ± 2.18[a]	3.27 ± 2.36[b]
Duodenal mitotic index	3 ± 0[a]	3 ± 1[a]	3 ± 2[a]	9 ± 2[b]
Colonic submucosal width (μ x 10^3)	2.45 ± 2.32[a]	2.0 ± 1.45[ab]	1.27 ± 1.16[ab]	1.18 ± 0.54[b]
Colonic mitotic index	2 ± 2[a]	2 ± 4[ab]	3 ± 1[ab]	5 ± 2[b]
Area of GALT* (cm^2)	72 ± 48[a]	108 ± 92[b]	168 ± 24[b]	164 ± 58[b]

+Values are medians with 25th percentiles
abValues with different letter superscripts are significantly different (p < 0.05)
*GALT = gut associated lymphoid tissue

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TABLE VIII. Median Gastric, Duodenal and Colonic Pathology Scores for Foals Treated with Phenylbutazone (PBZ), PBZ and Ranitidine, PBZ and Sucralfate, or Normal Saline for Ten Days

	PBZ	PBZ and Ranitidine	PBZ and Sucralfate	Control
Gastric epithelial necrosis	3 ± 1[a+]	1 ± 1[b]	1 ± 1[b]	0 ± 0[b]
Gastric mucosal inflammation	3 ± 2[a]	1 ± 1[ab]	1 ± 1[ab]	0 ± 0[b]
Gastric mucus score	2 ± 1[a]	1 ± 1[a]	0 ± 1[b]	0 ± 0[b]
Duodenal epithelial necrosis	2 ± 2[a]	2 ± 1[a]	2 ± 1[a]	0 ± 0[b]
Duodenal mucosal inflammation	1 ± 1[a]	1 ± 0[ab]	1 ± 1[ab]	0 ± 1[a]
Colonic epithelial necrosis	3 ± 1[a]	1 ± 1[b]	1 ± 0[b]	0 ± 0[c]
Colonic mucosal inflammation	2 ± 1[a]	1 ± 1[b]	1 ± 0[bc]	0 ± 0[c]
*GALT depletion/necrosis	2 ± 2[a]	1 ± 1[b]	0 ± 0[b]	0 ± 1[b]
Large intestinal ulceration	0 ± 0[a]	0 ± 1[b]	0 ± 0[b]	0 ± 0[b]

+Values are medians with 25th percentiles
abcValues with different letter superscripts are significantly different (p < 0.05)
*GALT = gut associated lymphoid tissue

Nonsteroidal anti-inflammatory drugs are associated with a decrease in the cellular production and secretion of bicarbonate, inhibition of mucosal cell turnover and repair, and an alteration in mucus structure. Another component of NSAID-induced mucosal injury is a focal ischemia caused by blood flow stasis in injured areas (28). Administration of a synthetic PGE$_2$ analogue has been shown to decrease PBZ-induced gastrointestinal ulceration in ponies (11).

The histamine H$_2$-receptor antagonists, cimetidine and ranitidine, are potent inhibitors of basal and cholinergic or pentagastrin-stimulated gastric acid secretion in humans and dogs (20). Studies in the horse have shown that ranitidine significantly reduces gastric acid secretion for 4 h following a single IV dose of 0.5 mg/kg BW (22). In this study, ranitidine was chosen in preference to cimetidine. Ranitidine suppresses gastric acid secretion for longer periods than cimetidine, thus allowing for a decreased frequency of administration. In addition, ranitidine does not inhibit the hepatic cytochrome P450 enzymes as does cimetidine (17,18). Phenylbutazone is metabolized by these hepatic enzymes (9).

Sucralfate, in addition to forming a protective barrier at an injured mucosal site, promotes mucosal synthesis and luminal release of PGE$_2$ which, in turn, stimulates mucus and bicarbonate production and epithelial cell restitution (16,18). Sucralfate provides additional cytoprotection by decreasing peptic activity by both adsorbing pepsin and buffering hydrogen ions (29).

Gastric ulceration was present in all foals treated with PBZ. However, the most severe gastric epithelial necrosis was seen in those foals treated with PBZ alone. In addition the GS mucus layer in both the PBZ and PBZ/ranitidine groups was absent. The ulcers present in the PBZ/sucralfate group appeared to be less active. In humans, both ranitidine and sucralfate allowed healing of NSAID-associated peptic ulcers despite continued administration of the NSAIDs (30). Although ranitidine provided partial protection against the toxic effects of PBZ on the gastric mucosa, sucralfate appeared to provide added protection by enhancing mucus production.

All three groups of foals receiving PBZ had marked duodenal villous atrophy, mucosal inflammation and a decrease in mitosis in the duodenal mucosal epithelium. This finding supports the results in a previous report (11) which suggested that mucosal atrophy is the primary lesion in PBZ toxicity. One explanation for the mucosal atrophy is that prostaglandins may be necessary for normal gastrointestinal mucosal cell turnover.

The presence of large intestinal lesions may also indicate that prostaglandins are necessary for the integrity of these organs. The foals in the PBZ group had the most severe cecal and colonic lesions and these were associated with diarrhea. The depletion and necrosis of GALT in the PBZ group was also a reflection on the severity of these lesions. Sucralfate and ranitidine appeared to provide some protection in the large intestine, although the reason for this is unclear. Sucralfate has known cytoprotective

properties (17,18), and ranitidine also may enhance mucosal defense mechanisms independent of its antisecretory effects (21).

Significant decreases in serum total protein, albumin and calcium were noted in all PBZ treated foals. This was most severe in the group given PBZ alone. The decrease in serum calcium is probably a reflection of the fact that 50% of calcium is albumin bound (31). In humans, an increase in small intestinal permeability to protein occurs within 12 h of NSAID administration (32) and is reversible (33). In horses, the specific sites of protein loss have not been identified (9), but in the present study ulcers and erosions in conjunction with villous atrophy could account for a protein-losing enteropathy. Alternatively, it is possible that changes in prostaglandin levels could lead to molecular protein loss in the absence of light microscopic morphological changes.

In this study, serum pepsinogen concentrations were not a sensitive indicator of gastrointestinal mucosal damage. Serum pepsinogen increases in humans with gastric or duodenal ulcers, in association with increased acid production (23). In a previous study foals with gastric and duodenal ulcers had elevations in serum pepsinogen concentrations, although there was considerable overlap with values obtained from control foals (34).

Ranitidine and sucralfate decreased the severity of oral cavity ulceration seen in PBZ treated foals, even though these drugs would not be expected to provide any protection in the oral cavity. Oral ulceration has been thought to be due to a local chemical irritant effect on the mucosa when PBZ is given orally (8). In the present study PBZ was administered intravenously, indicating systemic mediation of the ulceration, probably via inhibition of prostaglandin synthesis.

Embolic pneumonia, probably the result of jugular thrombophlebitis, was present in two foals from each of the PBZ and PBZ/ranitidine groups. These foals, together with two other PBZ group foals that had severe large intestinal lesions and septic peritonitis, showed an increase in immature neutrophil counts on TD 5 which persisted until TD 10.

No significant renal lesions were seen. In humans, it is suggested that NSAID-induced renal lesions are primarily seen in patients with dehydration following preexisting renal disease or decreased renal perfusion secondary to other conditions such as congestive heart failure and hypovolemia (36,37). In a retrospective study of 16 horses in which renal papillary necrosis was found at necropsy, it was determined that almost all had been treated with NSAIDs and had been dehydrated or their water intake had been compromised during the treatment (37). In the present study, none of the foals was clinically dehydrated until late in the treatment period and none had lesions of renal papillary necrosis.

The liver lesions seen in six of the foals were probably present prior to commencement of the study, since elevations in aspartate aminotransferase and gamma-glutamyl transferase were seen in four of these foals on TD 0. The cause of these lesions was not determined.

We conclude that phenylbutazone administered to foals at a dosage of 10 mg/kg/day for ten days is a suitable model for studying NSAID-induced ulceration. The histamine H_2-receptor antagonist, ranitidine and sucralfate, provided partial protection against the clinical, clinicopathological and pathological manifestations of phenylbutazone toxicity. Sucralfate was superior to ranitidine in that no foal from this group developed diarrhea, their gastric ulcers appeared less active, and a normal mucus covering was present in the GS.

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