Pathophysiologic effects of phenylbutazone on the right dorsal colon in horses

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Objective—To determine pathophysiologic effects of phenylbutazone on the equine right dorsal colon (RDC).

Animals—12 healthy adult horses.

Procedures—A controlled crossover observational study was conducted. Clinical and serum variables, colonic inflammation (histologic grading), and measurement of myeloperoxidase (MPO) activity, malondialdehyde (MDA) and prostaglandin E_2 (PGE_2) concentrations, ingesta volatile fatty acid (VFA) content, and arterial blood flow in the RDC were evaluated for a 21-day period in horses administered phenylbutazone (8.8 mg/kg, PO, q 24 h) or a control substance.

Results—Data from 8 horses were analyzed. Plasma albumin concentrations decreased significantly from days 10 to 21 during phenylbutazone treatment, compared with results during the same days for the control treatment. Phenylbutazone treatment caused neutropenia ($< 3.0 \times 10^3$ cells/μL). No other clinical or hematologic abnormalities were detected for phenylbutazone or control treatments. Two horses developed colitis while receiving phenylbutazone. No significant differences were detected in the RDC between phenylbutazone and control treatments for MPO activity, MDA and PGE_2 concentrations, and histologic evidence of inflammation. Arterial blood flow in the RDC was significantly increased during phenylbutazone treatment, compared with values for the control treatment. Differences were identified in VFA production during phenylbutazone treatment, compared with the control treatment, with a decrease in acetic acid concentrations over time.

Conclusions and Clinical Relevance—Prolonged phenylbutazone administration caused hypoalbuminemia, neutropenia, changes in RDC arterial blood flow, and changes in VFA production. Veterinarians should monitor serum albumin concentrations and neutrophil counts and be cautious when making dosing recommendations for phenylbutazone treatment of horses. (*Am J Vet Res* 2008;69:1496–1505)

Phenylbutazone, an NSAID, is one of the most commonly used drugs for treatment of equine athletes with signs of musculoskeletal pain,[1] and it is believed to be reasonably tolerated in horses when administered at the recommended dosage and dosing interval. In 1979 and again in 1980, 2 researchers separately reported[2–4] that standard administration of this drug to horses (4 to 8 mg/kg/d) can cause adverse effects, including gastric ulcers, renal dysfunction, and inflammation and ulcer-

ation of the mucosa of the large colon (ie, right dorsal colitis). These effects have subsequently been reported by several other groups.[4–7] The specific time frame and mechanism of action of phenylbutazone toxicosis in equids remain unclear, especially as it relates to doses at the higher end of the recommended dosing regimen (> 8 mg/kg/d for > 48 to 96 hours).[8]

Focal or diffuse chronic right dorsal colitis in horses is often characterized by an extensive disturbance of microcirculation in combination with signs of local inflammation (recruitment of neutrophils) and a subsequent systemic inflammatory response.[4,6,7,9] Although the specific pathway has not been determined in horses, it was an objective in the study reported here to determine whether colonic blood flow would be affected when horses are treated with phenylbutazone for

ABBREVIATIONS	
MPO	Myeloperoxidase
PGE_2	Prostaglandin E_2
RDC	Right dorsal colon
TMB	3,3′5,5′-tetramethylbenzidine
VFA	Volatile fatty acid

a long duration. We hypothesized that colonic blood flow would be decreased in horses during phenylbutazone treatment, compared with blood flow during treatment with a control substance, because inflammation, necrosis, and (in some cases) ischemia of the RDC are evident in horses affected with right dorsal colitis.

General disagreement exists about the dietary management of horses with suspected phenylbutazone-associated right dorsal colitis. Although some veterinarians recommend limiting dietary intake (feeding minimal or no hay or grain) to horses affected with right dorsal colitis to rest the intestines, others recommend feeding a high-roughage diet to stimulate production of specific short-chain VFAs (especially butyric acid) on the basis that increasing butyric acid content in the intestinal lumen is associated with protection of the intestinal mucosa in mice and humans.[10–12] Therefore, a specific objective of the study reported here was to assess VFA production in the RDC during phenylbutazone treatment.

The overall objective of the study reported here was to more specifically elucidate pathophysiologic mechanisms associated with administration of phenylbutazone to adult horses over a longer period (ie, > 2 weeks), compared with results for administration of a control substance. We hypothesized that administration of phenylbutazone to healthy horses at a standard dosing rate and duration would cause a decrease in blood flow in the RDC, inhibit mucosal PGE_2 concentrations, and alter production of VFAs. The specific objectives of the study were to evaluate clinical and clinicopathologic variables (signs of abdominal discomfort, degree of anorexia, fecal character, frequency of defecations, weight gain or loss, and evidence of fever) during serial clinical examinations in horses treated with phenylbutazone for 3 weeks; determine hematologic changes in horses administered phenylbutazone and a control substance; measure tissue inflammation by determining tissue hyperemia and edema via enumeration of tissue granulocytes by use of histologic assessment of mucosal biopsy specimens as well as by measurement of MPO activity, malondialdehyde concentrations, and mucosal PGE_2 concentrations; measure intraluminal production of VFAs in the RDC; and measure arterial blood flow in the RDC by use of ultrasonic flow probe techniques.

Materials and Methods

Animals—Twelve adult Thoroughbred or Quarter Horse–crossbred horses were used in the study. Horses were between 3 and 15 years of age and weighed between 400 and 550 kg. Horses were chosen from a pool of research horses maintained in facilities accredited by the Association for Assessment and Accreditation of Laboratory Animal Care International. Horses were considered healthy and free of gastrointestinal tract disease on the basis of results of physical examination, a CBC, and serum biochemical analysis and information in their preventative health history.

Horses were housed in box stalls in an enclosed, appropriately ventilated pole building throughout the study. During the study, horses were exercised by hand-walking for at least 20 minutes once daily. Horses were fed a diet of high-quality Bermuda grass hay (3% of to-

tal body weight or 13.5 kg of hay/450 kg of body weight daily) and pelleted 10% crude protein feed (0.8% of total body weight or 3.6 kg of feed/450 kgof body weight daily) and were allowed ad libitum access to tap water. The study was approved by the Louisiana State University Institutional Animal Care and Use Committee.

Horses were dewormed with moxidectin (0.4 mg/kg, PO) 6 weeks prior to the study. A larvacidal dosing regimen of fenbendazole (10 mg/kg, PO, q 24 h for 5 consecutive days) was administered 2 weeks after moxidectin (ie, 4 weeks prior to the study). All horses were seronegative for equine infectious anemia and were vaccinated against encephalitis viruses (eastern and western equine encephalomyelitis viruses and West Nile virus), influenza, tetanus, and rhinopneumonitis at least 2 months prior to the beginning of the study. A single fecal sample was obtained for *Salmonella* culture of each horse prior to the start of the study as a screening test for salmonellae shedding.

Fistula creation and cannula implantation in the RDC—One or 2 horses were enrolled in the study at a time; the study was conducted during an 18-month period. Each horse underwent 2 surgeries for creation of a fistula and for instrumentation.

Fistulas were created in the RDC in a 2-stage procedure.[13] For the first stage, each horse was anesthetized and positioned in dorsal recumbency. A 20-cm skin incision was made caudal to and parallel with the curvature of the 16th rib. Muscle layers and the external periosteum of the 16th rib were sharply incised. The distal third of the rib was resected, the internal periosteum was incised, and the abdominal cavity was entered. A portion (6 to 10 cm in diameter) of the muscular body wall was resected to create a site for adhesion formation to the colon. The RDC was then identified, and a similar area (6 to 10 cm in diameter) was circumferentially sutured to the site of the surgically created defect in the muscular body wall. Synthetic absorbable suture (polyglactin 910,[a] size 0) was used in a minimum of 3 layers (peritoneum to colon, intercostal muscle to colon, and external abdominal oblique and cutaneous coli muscles to colon). The seromuscular and submucosal layers, but not the mucosa, were incorporated into the sutures. Subcutaneous tissue and skin were closed in a routine manner over the exposed colon. Horses were allowed to recover from anesthesia and maintained in a box stall for 2 weeks to allow a strong adhesion to form between the colon and body wall.

The second stage of the procedure was performed with each horse sedated by administration of detomidine (0.01 mg/kg, IV) and butorphanol tartrate (0.01 mg/kg, IV) and restrained in a standing position. The area in which the RDC was adhered to the body wall was identified by use of transabdominal ultrasonography. After each horse was sedated, skin at the site of the fistula was aseptically prepared and locally infiltrated with anesthetic. Then a section (2 to 3 cm in diameter) of skin and fibrous tissue was resected over the area of the adhered colon. The wall of the RDC was sharply incised, and a silastic intestinal cannula (internal diameter, 2.5 cm)[b] was inserted and secured. A metal clamp was applied to the end of the cannula that exited the colon; the clamp was tightened to occlude the cannula

lumen. The cannula was removed and cleaned daily (and more often as needed) to prevent occlusion. Time to clean the cannula was < 5 minutes, and the cannula was then immediately replaced.

Implantation of the blood flow probe—During the first-stage surgery for creation of the fistula in the RDC, a 3-mm perivascular ultrasonic blood flow probe[c] was implanted around the mesocolonic artery of the RDC. Briefly, a 2-cm segment of the mesocolonic artery was isolated, and the blood flow probe was placed and sutured to the surrounding mesocolonic tissue by use of a synthetic nonabsorbable polypropylene suture[d] (size 3-0). The peritoneal serosa was sutured with polyglactin 910[a] (size 3-0) in a simple continuous pattern. Cruciate sutures (synthetic, nonabsorbable polypropylene suture[d]; size 3-0) were used to affix the cable for the probe to the mesocolonic tissue. The cable exited the body wall at a site distant from the location of the adhesion of the RDC to the body wall (for the fistula-canula). The cable was tunneled subcutaneously for a minimum of 5 cm before it exited through the skin. The probe cable was secured to the skin with size 3-0 polypropylene by use of a Chinese finger trap technique.[14] The cable and adapter were protected with padding and by suturing a stent bandage to the skin. The adapter was connected via a long extension cable to the flow meter to measure colonic blood flow before and serially after administration of treatments.

Experimental procedures—After recovery from anesthesia and the second surgery to create the fistula and insert the cannula, horses were allowed to acclimate for at least 2 days in a box stall. Horses were randomly assigned to 2 groups (6 horses/group). Horses in one of the groups received phenylbutazone as the first treatment, and horses in the other group received a control substance (corn syrup) as the first treatment. After a washout period, the horses received the alternate treatment. Phenylbutazone[e] (8.8 mg/kg) or the control substance (a volume of corn syrup equivalent to the volume of phenylbutazone [approx 4 mL/d]) was administered orally to the respective horses. Treatments were divided into 2 equal doses and administered at a mean ± SD interval of 12 ± 1 hour. The dose of corn syrup was considered sufficiently small that it would not affect VFA concentrations in the large colon. The investigators were aware of the treatment administered to each horse. Treatment continued for 21 days unless signs of toxicosis became clinically apparent, which required discontinuation of the study for affected horses.

Each horse remained in its stall and was fed the same diet during the washout period of 3 to 4 weeks. A longer washout period would have been preferable to allow additional time for healing of the colonic mucosa in phenylbutazone-treated horses; however, our experience with the flow probe revealed that the probe often lost functionality after 2 or 3 months; thus, we did not want to risk the likelihood that we would not be able to collect data on blood flow for the second treatment during the study.

Physical examinations of each horse were performed daily by a veterinarian, veterinary technician, or both. Instrumentation and cannula sites were maintained and cleaned daily or more frequently when necessary. Two horses were treated with potassium penicillin G (22,000 U/kg, IV, q 6 h) and gentamicin sulfate (6.6 mg/kg, IV, q 24 h) because signs of focal cellulitis associated with cannula or probe placement were detected (1 horse required antimicrobial administration during the treatment period, and the second horse required antimicrobial administration during the washout period).

Clinical and laboratory evaluations—Physical examinations (rectal temperature, heart rate, respiratory rate, capillary refill time, abdominal auscultation, and demeanor) were performed on horses once daily throughout the study. In addition to objective variables for the physical examination, fecal character,[15] frequency of defecation, signs of abdominal discomfort, and degree of anorexia were recorded. Abdominal discomfort and degree of anorexia were assessed daily by use of colic assessment flow sheets from the Louisiana State University Veterinary Teaching Hospital and Clinic. Abdominal discomfort and anorexia were each subjectively graded on a scale of 0 to 3 (0, no signs of abdominal discomfort or anorexia; 1, signs of mild discomfort or anorexia; 2, signs of moderate discomfort or anorexia; and 3, signs of severe discomfort or anorexia).

Blood samples were collected via jugular venipuncture into 2 tubes (1 contained no anticoagulant, and the second contained EDTA as an anticoagulant) twice weekly beginning on the first day of treatment (day 0) and continuing through day 21 of each study (ie, days 4, 7, 10, 13, 16, 19, and 21). The BUN concentration and plasma concentrations of electrolytes, creatinine, albumin, and total protein were determined by use of standard biochemical analysis with a commercially available automatic clinical chemistry analyzer.[f] The PCV and total WBC count were calculated by use of a commercially available clinical hematology analyzer.[g] A commercial Wright-Giemsa stain[h] was used to stain cells, and differential cell counts were performed by counting 100 cells by use of a light microscope (1,000✕ magnification).

Assessment of blood flow—Arterial blood flow in the RDC was monitored twice daily (approx 2 and 3 hours after the morning grain ration was ingested), and the mean value was recorded. Blood flow monitoring was conducted on the first day of each treatment (day 0) and twice weekly through day 21 (ie, days 4, 7, 10, 13, 16, 19, and 21).

Endoscopic evaluation and sample collection—Gross evaluation of the mucosal surface of the RDC was followed by collection of 3-mm biopsy specimens. Evaluations and biopsy procedures were performed by use of a flexible fiberoptic endoscope[i] with a 155-cm, 3-mm flexible alligator jaw endobiopsy forceps inserted via the indwelling cannula. Sample specimens included the mucosa, submucosa, laminae propria, and muscle layers. Specimens were collected beginning on day 0 of each experiment and twice weekly through day 21. Twelve specimens were obtained from closely adjacent sites at each sample collection, which yielded 3 specimens for histologic assessment and 9 specimens for bio-

chemical assays. Samples were obtained with the aid of a visual subjective grid system whereby sample sites were located close to but not at the same site as the preceding sample sites. Location of sample sites was recorded for each collection so that biopsy specimens were not obtained from previous sampling sites. Specimens for histologic examination were placed in neutral-buffered 10% formalin. Specimens for biochemical assays were placed in aluminum foil, flash-frozen in liquid nitrogen, and stored at –70°C. Three frozen specimens from each respective time-treatment period from each horse were pooled to provide a larger sample size for analysis of MPO activity, malondialdehyde concentration, and PGE_2 concentration. A sample of ingesta (20 mL) was collected and processed for VFA analysis.

Histologic examination—Each tissue specimen was fixed in neutral-buffered 10% formalin for 24 hours. Specimens were then embedded in paraffin, cut in sections at a thickness of 5 μm, and stained with H&E for examination by use of light microscopy. Sections of RDC mucosa from each time period and treatment group were examined via light microscopy by a board-certified veterinary pathologist (TWM), who was unaware of the sample treatment. Tissue samples were subjectively graded with respect to edema, focal hemorrhage, and infiltration of granulocytic cells by use of a scoring system.[16] The scale ranged from 0 to 3 (0, no hemorrhage or edema observed; 1, slight to mild hemorrhage or edema; 2, moderate hemorrhage or edema [extensive but did not distort normal architecture]; and 3, marked to severe hemorrhage or edema [distorted normal architecture]). The extent of infiltration of granulocytic cells associated with inflammation was subjectively assessed, and each section was assigned a grade of 0 to 3 (0, normal tissue with no or few granulocytes [eosinophils and neutrophils]; 1, minimal inflammation with minimal to low numbers of scattered granulocytes; 2, moderate inflammation with a noticeable number of granulocytes with foci in the mucosa; and 3, marked inflammation with many granulocytes with foci in the submucosa and vessels).[16]

MPO assay—Activity of MPO was used as a reflection of granulocyte activity and was determined by use of a modification of the Grisham method in which the enzyme catalyzes the oxidation of TMB via hydrogen peroxide to yield a blue chromogen with a maximum wavelength of 655 nm.[17,18] Tissue specimens were thawed, weighed, and homogenized in potassium phosphate buffer. The homogenate was mixed with a detergent to disrupt granules and solubilize peroxidase enzymes. An aliquot of homogenate was added to a mixture of sodium phosphate buffer and TMB. The reaction was initiated by adding hydrogen peroxide, and the change in absorbance at 655 nm was measured during a 2-minute period. One unit of MPO activity was defined as that degrading 1 μmol of peroxide/min at 25°C.

Lipid peroxidation—Malondialdehyde synthesis was used as a marker for lipid peroxidation of tissues.[19] Samples were homogenized in 1.15% KCl and mixed with SDS, thiobarbituric acid, and potassium phosphate buffer; solutions were then heated for 60 minutes in a water bath at 95°C. Water and *n*-butanol pyridine were added, the mixture was centrifuged, and the organic layer (containing thiobarbituric acid–reactive malondialdehyde) was evaluated spectrophotometrically.

PGE_2 assay—Methods for tissue extraction and purification have been described in detail.[20] Briefly, tissues were thawed and homogenized in cold 80% ethanol. Sample extracts were loaded onto C-18 columns,[j] and the eluant was obtained for measurement of PGE_2 concentrations by use of a radioimmunoassay.[k]

VFA analysis—A sample (20 mL) of ingesta was collected from the RDC, and an aliquot (4 mL) was collected from a homogenized sample of the colonic liquor. The 4-mL sample of colonic fluid was mixed with 1 mL of 25% (wt/wt) metaphosphoric acid containing 2 g of 2-ethylbutyric acid/L, which was used as an internal standard for VFA quantification. The mixture of colonic fluid and metaphosphoric acid was then centrifuged at 30,000 $\times$ g for 25 minutes. Concentrations of VFAs were measured by use of gas-liquid chromatography.[21]

Statistical analysis—Analysis was performed by use of a commercially available software program.[l] Values for physical examination variables, total plasma protein concentration, plasma albumin concentration, PCV, total WBC count, neutrophil count, BUN concentration, plasma creatinine concentration, histologic grade, blood flow in the RDC, and intraluminal VFA concentrations were evaluated for normality by use of the Shapiro-Wilk method with the null hypothesis rejected at $\alpha = 0.05$. Normally distributed data were analyzed by use of a mixed-effect general linear model with repeated measures. Predetermined post hoc comparisons were made by use of least squares means with a Bonferroni correction. Values of $P < 0.05$ were considered significant for all tests.

Results

Clinical variables—Four horses were removed from the study because of medical reasons. Two horses developed acute necrotizing enterocolitis at 7 and 10 days, respectively, of phenylbutazone administration; these horses required hospitalization and several days of supportive care. One horse developed septic peritonitis believed to be associated with the exit site of the blood flow probe; this horse required hospitalization and prolonged antimicrobial treatment. The final horse was euthanatized after signs of unrelenting abdominal pain were detected prior to the onset of the study. Necropsy of that horse revealed enterolith obstruction of the large colon. No data were used from the 4 horses that were removed from the study.

No significant differences in results were detected between the phenylbutazone and control treatments for vital parameters (rectal temperature, heart rate, respiratory rate, and capillary refill time) throughout the study. Similarly, demeanor, degree of anorexia, signs of abdominal comfort, frequency and character of borborygmi, fecal character, and frequency of defecation did not differ significantly between treatments throughout the study.

Clinicopathologic findings—A significant decrease in plasma albumin concentrations was detected from days 3 to 21 during administration of phenylbutazone, compared with the concentration on the same days during administration of the control treatment (**Figure 1**). The reference range of plasma albumin concentration in horses for the laboratory was 3.0 to 3.9 g/dL, with slight fluctuations in clinically normal horses associated with hydration status. Changes in plasma albumin concentrations for specific horses ranged from a decrease of 0.1 to 1.0 mg/dL, with a mean decrease of 0.41 mg/dL in horses during phenylbutazone administration. There was no significant decrease in total plasma protein concentration between treatments. No differences in plasma concentrations of sodium, potassium, chloride, or creatinine or BUN concentrations were detected between treatments (data not shown).

Neutrophil counts of horses during administration of the control treatment were within the reference range (2.5 $\times 10^3$ cells/μL to 6.2 $\times 10^3$ cells/μL) and did not change significantly in each horse during the 21-day period (**Figure 2**). However, neutrophil counts decreased significantly during phenylbutazone treatment, ranging from a decrease of 41% to a decrease of 85% (mean decrease, 52%). Two of 8 horses had circulating band cells during phenylbutazone treatment (one beginning on day 9 and the second beginning on day 12; data not shown). Neutrophil counts were decreased in the first 3 to 6 days after initiation of phenylbutazone treatment and were significantly different from counts for the control treatment at 10, 15, and 20 days after initiation of treatment. Although neutrophil counts differed significantly between the 2 treatments, the overall total WBC count remained within the reference range for the laboratory and did not differ significantly between treatments.

Assessment of mucosal inflammation—Histologic assessment of mucosal biopsy specimens revealed no significant differences in cellular infiltration, mucosal edema, or tissue necrosis between treatments on the basis of results for the standard scoring system (**Figure 3**). In addition, no significant differences between treatments were detected in mucosal tissues for MPO activity, malondialdehyde concentration, and PGE_2 concentration (data not shown).

Arterial blood flow in the RDC—Arterial blood flow in the RDC had a wide range of values between treatments as well as within individual horses (data not shown). Arterial blood flow in the RDC was significantly higher for the phenylbutazone treatment at days 16 and 21, compared with the blood flow for the control treatment on those same days (**Figure 4**). There was a gradual overall decrease in blood flow over time for both treatments.
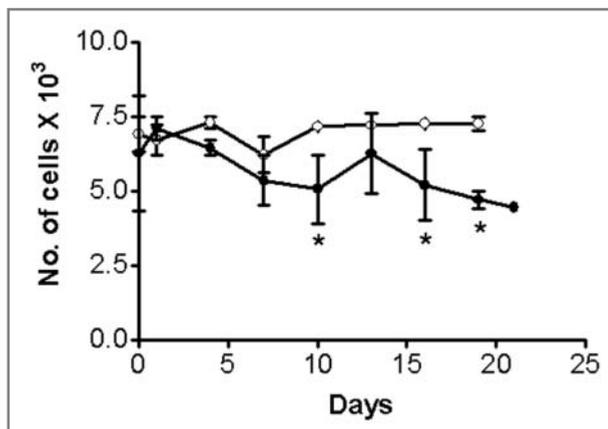

Figure 2—Mean ± SEM neutrophil count during daily administration of phenylbutazone (black circles) or a control substance (white circles) to 8 horses for 21 days. Data were not available for the control treatment on day 21. *See* Figure 1 for remainder of key.
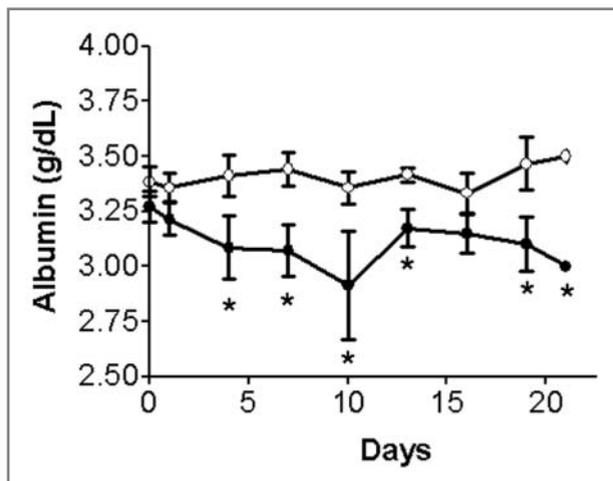

Figure 1—Mean ± SEM plasma albumin concentrations during daily administration of phenylbutazone (8.8 mg/kg, PO [black circles]) or a control substance (an equivalent volume of corn syrup [white circles]) to 8 horses for 21 days. The daily dose was equally divided and administered at a mean ± SD interval of 12 ± 1 hour. Samples for measurement of albumin concentrations were obtained on day 0 (first day of administration) and twice weekly through day 21. *Within a day, value differs significantly (*P* < 0.05) between treatments.
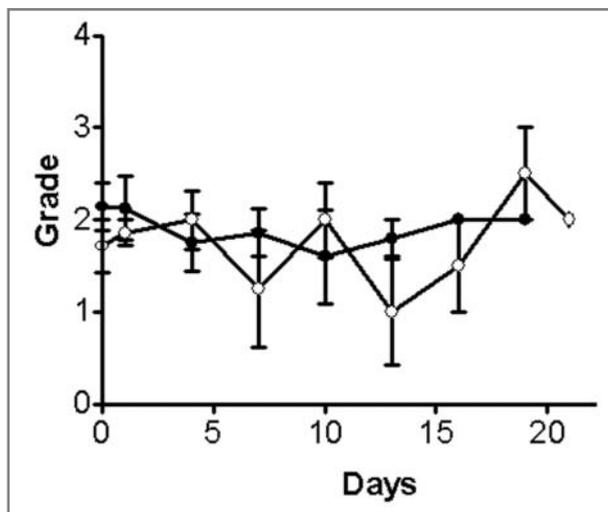

Figure 3—Mean ± SEM histologic grade of tissue sections of the RDC obtained during daily administration of phenylbutazone (black circles) or a control substance (white circles) to 8 horses for 21 days. Tissue samples were subjectively graded with respect to edema, focal hemorrhage, and infiltration of granulocytic cells by use of a scoring system that ranged from 0 to 3 for each characteristic (0, no changes; 1, mild changes; 2, moderate changes; and 3, severe changes). Values differed significantly (*P* < 0.05) between treatments. *See* Figure 1 for remainder of key.
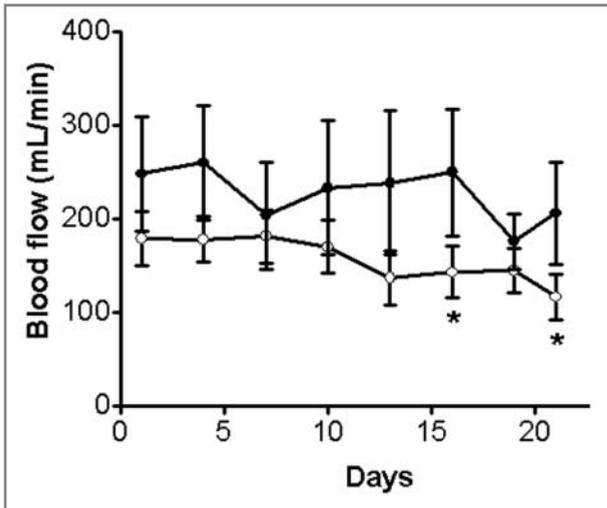
Figure 4—Mean ± SEM arterial blood flow in the RDC during daily administration of phenylbutazone (black circles) or a control substance (white circles) to 8 horses for 21 days. *See* Figure 1 for remainder of key.
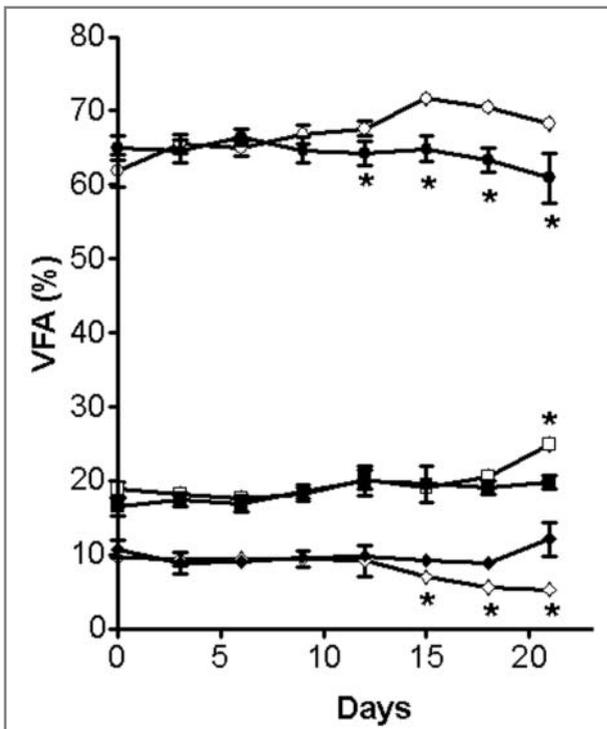

Figure 5—Mean ± SEM percentage change in VFAs (acetic acid [circles], butyric acid [diamonds], and propionic acid [squares]) in samples of ingesta obtained from the RDC during daily administration of phenylbutazone (black symbols) or a control substance (white symbols) to 8 horses for 21 days. *Within a day for each VFA, value differs significantly ($P < 0.05$) between treatments. *See* Figure 1 for remainder of key.

VFA analysis—Butyric acid concentrations in the ingesta samples collected from the RDC did not change over time during phenylbutazone treatment, whereas they decreased significantly on days 12 to 21 during the control treatment (**Figure 5**). Acetic acid concentrations decreased during phenylbutazone treatment. Propionic acid concentrations remained unchanged during phenylbutazone treatment.

Discussion

The purpose of the study reported here was to examine potential adverse systemic effects and to serially evaluate local pathophysiologic outcomes in the RDC during a 21-day period in which phenylbutazone was administered orally (8.8 mg/kg/d) to adult horses. Our hypothesis was that administration of phenylbutazone to healthy horses at a standard dosing rate and duration would cause a decrease in arterial blood flow in the RDC, inhibit mucosal PGE_2 concentrations, and cause a change in production of VFAs, all of which would lead to mucosal damage and inflammation with subsequent hypoproteinemia and hypoalbuminemia. Long-term was defined in this study as treatment for > 5 days, and we used a 21-day treatment regimen. Clinical and clinicopathologic variables, colonic tissue inflammation, colonic blood flow, and luminal VFA production were serially evaluated and analyzed.

In the study reported here, all horses had total plasma protein and albumin concentrations within the reference range at the start of each treatment period. Hypoalbuminemia (albumin concentration < 3.0 mg/dL) was detected during phenylbutazone treatment as early as 3 days after initiation of phenylbutazone administration, and albumin concentrations continued to decrease during the 21 days of phenylbutazone treatment in 5 of 8 horses. In 2 horses that developed acute colitis and were removed from the study, severe hypoproteinemia and hypoalbuminemia were evident on days 5 and 7 of phenylbutazone treatment, respectively. Horses had total plasma protein and albumin concentrations within the reference range throughout the control treatment.

Hypoalbuminemia is used anecdotally as an indicator of dysfunction of the intestinal mucosal barrier, such as colitis-associated infiltration of inflammatory cells into the mucosa, edema of the mucosa, or necrosis of mucosal cells.[22–24] The findings in the study reported here indicated that plasma albumin concentration was the first clinicopathologic variable to decrease during phenylbutazone treatment. Plasma albumin concentration may be one of the most sensitive hematologic variables to evaluate when monitoring a horse for the possibility of chronic NSAID-associated colitis and was more sensitive than measurement of total plasma protein concentration alone. However, because urine protein analyses were not included in the study design, the possibility of albumin loss associated with renal damage cannot be completely ruled out. However, because BUN and plasma creatinine concentrations remained within the respective reference ranges during the phenylbutazone and control treatments, renal albumin loss was less likely to have been a cause.

Neutropenia was also a hematologic variable that proved to be important in this study during phenylbutazone treatment because horses were becoming neutropenic beginning as early as 3 and 5 days after the initiation of treatment. The NSAIDs, such as phenylbutazone, have often been implicated as a cause of acute colitis or acute necrotizing colitis in horses; however, this has been difficult to prove because there are often other confounding factors, including other medications associated with treatment of these horses.[6,9] Two horses in the study reported here developed severe colitis dur-

ing phenylbutazone treatment, which lends clinical evidence to support this hypothesis.

No significant histologic differences were detected between the 2 treatments. This may have been attributable to the low number of horses ultimately available for analysis or the possibility that the histologic grading system was not sufficiently sensitive to detect differences between the 2 treatments. In addition, variability within each horse with regard to the response to phenylbutazone treatment and, in particular, the exclusion of 2 horses because of signs of acute necrotizing colitis resulted in the unavailability of the most severely affected colonic tissues for analysis. There was ample opportunity for substantial variability within each horse in this study. It should be mentioned that the technique used in our study (use of multiple biopsy specimens collected throughout the treatment periods) could have caused colonic damage and may have been a source for the neutropenia and albumin abnormalities. However, because horses were subjected to the same biopsy techniques during administration of phenylbutazone and the control treatment and results during administration of the control treatment did not reveal neutropenia or hypoalbuminemia, this was not considered to be a problem.

Results from this study that apparently link colonic mucosal abnormalities and adverse systemic effects support the concept that phenylbutazone causes changes in the equine gastrointestinal mucosal barrier that are not necessarily associated with specific identifiable pathologic changes. The fact that NSAIDs act differently in their ability to induce mucosal injury in different regions of the gastrointestinal tract in accordance with their pharmacologic properties, formulation, and the type of mucosal injury suggests that other mechanisms may play a bigger role in NSAID-associated toxicosis. Such mechanisms may include mucosal restitution (epithelial healing process whereby adjacent injured mucosa is covered by responding epithelial cells) via molecular processes involving integrins, kinases, and growth factors.[25] Other important processes could include interepithelial tight junctions and the paracellular space, which is suggested by measures of barrier function such as transepithelial electrical resistance.[25,26] Additional roles may be played by subepithelial immunocyte populations, including neutrophils, eosinophils, and mast cells.[18,26] The subsequent neutropenia is most likely associated with disruption of the mucosal barrier and absorption of gram-negative organisms and their by-products, which results in endotoxin absorption and dissemination into the systemic circulation.[27]

Focal or diffuse chronic right dorsal colitis in horses may be associated with an extensive disturbance of microcirculation and signs of systemic inflammatory response syndrome, as postulated in other reports.[4,6,7] However, this specific pathway has not been determined in horses. It was our intention to determine whether blood flow in the RDC would be affected when horses are treated for a long duration with phenylbutazone. Our hypothesis was that blood flow in the RDC would decrease during phenylbutazone treatment, compared with blood flow during the control treatment, because necropsy of horses with right dorsal colitis has revealed

inflammation, necrosis, and (in some cases) evidence of ischemia.[6,7,9] However, in our study, blood flow in the RDC was significantly increased at only 2 time points (days 16 and 21) during phenylbutazone treatment, compared with blood flow on those same days during the control treatment. In addition, there was a slight but gradual overall decrease in blood flow in the RDC during both treatments, with a greater decrease during the control treatment, which may have accounted for the differences between treatments. Another important finding was that there was extensive variability in blood flow in the RDC within each horse as well as between treatments.

Increased blood flow in the RDC during phenylbutazone treatment, compared with blood flow during the control treatment, could have indicated a reflection of a generalized diffuse colonic inflammatory response with subsequent vasodilatation as well as the possibility of angiogenesis. Because there was a gradual decrease in blood flow over time during both treatments, this could have been associated with the chronic instrumentation technique used in this study; therefore, these results are inconclusive. Additional research would be required to address the cause of these changes.

Angiogenesis secondary to chronic intestinal inflammation is reported in humans and domestic animals with experimentally induced inflammatory bowel disease.[28–30] Studies[28,29] in mice with experimentally induced inflammatory bowel disease have revealed that experimentally induced colitis is characterized by angiogenic inflammation that contributes to the development and sustenance of the experimentally induced colitis. Furthermore, these studies[19,28] provide compelling evidence that increased leukocyte recruitment is required for angiogenic stimulation during chronic colitis. Although the study reported here did not detect significant differences in the colonic inflammatory response between the 2 treatments (ie, no difference in tissue MPO activity, malondialdehyde concentration, PGE_2 concentration, or histologic grade), there is evidence in other studies[4,7,9,18,26] to suggest that right dorsal colitis in horses may involve tissue recruitment of immunocytes and persistent inflammatory cell infiltration, thus making a case for angiogenic stimulation in horses with right dorsal colitis. The angiogenic response detected in animals with experimentally induced colitis may contribute to chronic inflammation, which likely enables a vicious cycle of disease activity, such as is evident in horses with NSAID-associated right dorsal colitis.

Increased angiogenesis would be likely in chronic inflammation, which could be in response to mucosal necrosis. However, this was not a finding in the horses of the study reported here. It is possible that the small number of horses included in this study was not sufficient for us to detect significant differences, and there was also within-horse variability confounded by the fact that 2 of the most severely affected horses had to be removed from the study for humane reasons. If these 2 horses had been allowed to remain in the study, it is possible that a significant association between the 2 variables would have been identified.

Concentrations of VFAs produced by intestinal contents during treatment with phenylbutazone and the

control substance were evaluated in this study because variations in VFA concentrations have been correlated with intestinal pathogenicity in other species.[10,11,26] It is important to determine the dynamics and potential interactions of intestinal bacteria in inflamed versus healthy intestines of horses because it may help explain pathophysiologic mechanisms. With this information, possible management practices involving administration of medications or probiotics or dietary management in horses with right dorsal colitis or other inflammatory conditions affecting the gastrointestinal tract could be devised.

Volatile fatty acids are an important product of colonic bacterial fermentation processes and are largely responsible for water absorption in the distal part of the colon in equids. Results from this study revealed that concentrations of acetic acid were significantly lower after 2 weeks of phenylbutazone treatment. Acetic acid concentrations continued to decrease throughout the third week during phenylbutazone treatment, whereas butyric and propionic acid concentrations remained unchanged during phenylbutazone treatment. It has been suggested in another study[26] that organic acids produced by anaerobic intestinal bacteria (as is the situation in the large colon of horses) contribute to the pathogenesis of colonic inflammation. More specifically, acetate, butyrate, and propionate cause potent cytotoxic effects (specifically apoptosis) in the colonic epithelial cells of clinically normal mice, whereas it has been suggested in other studies[10,11,26] that butyrate in the intestinal lumen may be useful as a novel treatment for inflammatory conditions of the distal part of the intestinal tract.

Results of the study reported here supported the hypothesis that phenylbutazone administration at a dose of 4.4 mg/kg every 12 hours causes adverse gastrointestinal tract effects that may begin as early as 3 days after the initiation of treatment. Even though there was not compelling evidence of inflammation in the tissues of the RDC, other mechanisms of intestinal albumin leakage involving other sites of the intestinal tract are likely. The fact that 2 horses developed acute colitis during phenylbutazone treatment may be important and provide additional possible support of this association, although this can only be speculative. Although the frequency of intestinal ulcers secondary to treatment with NSAIDs (such as phenylbutazone) is unknown, it is likely that animals with only moderate to severe ulcers are recognized and treated. However, it is possible that many cases of phenylbutazone toxicosis may go undetected via clinical assessment, as suggested in this study by the lack of differences in clinical assessments but significant differences in plasma albumin concentrations, neutrophil counts, and VFA concentrations.

In a controlled prospective study,[31] investigators determined that IV administration of phenylbutazone (4.4 mg/kg, q 8 h for 12 days) caused the most severe damage to the gastrointestinal tract (stomach, small intestines, and large colon), compared with results after IV administration of ketoprofen (2.2 mg/kg, q 8 h for 12 days) or flunixin meglumine (1.1 mg/kg, q 8 h, for 12 days). The doses administered in that study are considerably greater than the doses routinely recommended for horses, but the results support the conclusion that phenylbutazone is generally more toxic than flunixin meglumine or ketoprofen when administered at these high doses. In a more recent study,[22] administration of phenylbutazone in combination with a second NSAID (flunixin meglumine), which is a fairly common practice for veterinary management of horses in the United States, caused horses to become hypoproteinemic after 5 days and to develop gastric ulcers, compared with results for horses that received no treatment or horses that were treated for 5 days with a low dose of phenylbutazone alone. Other clinical studies[32–34] have revealed that treatment with moderate to high doses of phenylbutazone (3.0 to 4.4 mg/kg, PO, q 12 h) was possibly related to stricture formation in the colon that required surgical resection, bypass surgery, or change to a strictly pelleted diet.

In humans, chronic inflammatory conditions secondary to NSAID treatment can lead to expression of specific genes and subsequent development of intestinal carcinoma[30]; this scenario has not yet been identified in horses, but it is possible and likely. The mechanism of action whereby NSAIDs lead to intestinal damage is via cyclooxygenase blockade and subsequent inhibition of eicosanoid formation. There is specific inhibition of the intestinal mucosal protective effect of PGE_2, which leads to mucosal ischemia, inflammation, and ulceration or erosion; however, the exact mechanism or mechanisms have yet to be elucidated. Alleviation of fever and pain by administration of phenylbutazone must be weighed against these possible adverse effects on local target tissues, specifically the large colon and stomach. It is difficult to diagnose right dorsal colitis because of the often vague clinical signs (partial to complete loss of appetite or weight and sporadic episodes of signs of abdominal pain, diarrhea, and loss of protein from the damaged intestines) and the remote location of the RDC. Treatment options are even more difficult to address because the pathophysiologic processes of right dorsal colitis are poorly understood.

Based on anecdotal evidence, treatment for horses with right dorsal colitis has mainly involved supportive care (discontinuation of NSAID administration and replacement of electrolytes, fluids, and protein) and empirical dietary modification; however, surgical resection of affected intestine has been successful in a few cases.[31–34] There is general disagreement regarding the dietary management of horses with right dorsal colitis. Although some veterinarians recommend limiting dietary intake (feeding minimal or no hay or grain) in horses affected with right dorsal colitis to rest the intestines, others recommend feeding a high-roughage diet to stimulate production of specific short-chain VFAs, especially butyric acid, which has been associated with intestinal mucosal protection in humans and some domestic animals with experimentally induced disease. However, other scientific evidence suggests the contrary, and increases in butyric acid concentrations may be detrimental or merely reflect a pathologic state.[35,36] Additional studies are warranted to determine the importance of VFA concentrations in the large intestines of healthy and diseased horses.

In the study reported here, phenylbutazone treatment caused hypoalbuminemia and neutropenia during the first few days (3 days) after initiation of treatment, changes in blood flow in the RDC, and alterations in luminal concentrations of VFAs. Although we were not able to confirm it in our study, these clinicopathologic changes are possibly the result of local changes in the colonic mucosa, including initiation of an inflammatory cascade, recruitment of intestinal immunocytes, dysfunction of the intestinal barrier, and absorption of endotoxin from gram-negative bacteria. However, it is equally likely that other segments of the intestine as well as other organs may have been involved in the changes. Intestinal angiogenesis and a subsequent increase in the blood flow in the RDC may have been secondary to complicated mechanisms associated with inflammation that we were unable to elucidate in this type of study format. Equine veterinarians should be extremely judicious and cautious when making dosing recommendations for phenylbutazone treatment. There is extreme variability within horses, and some horses are unable to tolerate even 5 days of treatment at this dose and administration frequency. Routine hematologic analysis beginning as early as 3 to 5 days after initiation of treatment should be performed, and the phenylbutazone dose should be decreased or phenylbutazone administration should be discontinued so that debilitating and life-threatening adverse effects of the gastrointestinal tract do not develop. Alternative NSAIDs with proven clinical efficacy and less adverse effects (ie, specific agents [such as firocoxib] that spare cyclooxygenase-1) are becoming more available for horses and should be considered.

a. Vicryl, Ethicon, Somerville, NJ.
b. Bar Diamond Inc, Parma, Idaho.
c. Transonics Inc, Ithaca, NY.
d. Prolene, Ethicon, Somerville, NJ.
e. Phenylzone, Schering-Plough Animal Health, Union, NJ.
f. AU640e, Olympus America Inc, Irving, Tex.
g. Baker Instruments System 900, Baker Instrument Co, Fort Collins, Colo.
h. Diff Quick stain set, Andwin Scientific, Canoga Park, Calif.
i. Fiberscope 60512 VG, Storz Endoscopy, Goleta, Calif.
j. Waters C-18 Sep Pak, Advanced Magnetics Inc, Cambridge, Mass.
k. PGE2 kits, Cayman Chemical, Ann Arbor, Mich.
l. GraphPad Prism, version 3.00 for Windows, GraphPad Software, San Diego, Calif.

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