Effects of phenylbutazone alone or in combination with flunixin meglumine on blood protein concentrations in horses

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Objective—To assess effects of treatment with phenylbutazone (PBZ) or a combination of PBZ and flunixin meglumine in horses.

Animals—24 adult horses.

Procedure—13 horses received nonsteroidal anti-inflammatory drugs (NSAIDs) in a crossover design. Eleven control horses were exposed to similar environmental conditions. Treated horses received PBZ (2.2 mg/kg, PO, q 12 h, for 5 days) and a combination of PBZ and flunixin meglumine (PBZ, 2.2 mg/kg, PO, q 12 h, for 5 days; flunixin meglumine, 1.1 mg/kg, IV, q 12 h, for 5 days). Serum samples were obtained on day 0 (first day of treatment) and day 5, and total protein, albumin, and globulin were measured.

Results—1 horse was euthanatized with severe hypoproteinemia, hypalbuminemia, and colitis during the combination treatment. Comparisons revealed no significant difference between control horses and horses treated with PBZ alone. There was a significant difference between control and treated horses when administering a combination of PBZ and flunixin meglumine. Correction for horses with values > 2 SDs from the mean revealed a significant difference between control horses and horses administered the combination treatment, between control horses and horses administered PBZ alone, and between horses receiving the combination treatment and PBZ alone. Gastroscopy of 4 horses revealed substantial gastric ulcers when receiving the combination NSAID treatment.

Conclusions and Clinical Relevance—Analysis of results of the study indicates the need for caution when administering a combination NSAID treatment to horses because the detrimental effects may outweigh any potential benefits. (Am J Vet Res 2006;67:398–402)

Nonsteroidal anti-inflammatory drugs are widely used by horse owners, trainers, and veterinarians. The practice of combining NSAIDs has been empirically used to enhance the analgesic properties of these drugs in performance horses. The effect of NSAID use in horses has been studied extensively, and the potential adverse effects of NSAIDs in the kidneys and gastrointestinal tract have been reported. Specific NSAIDs have varying degrees of toxicity. The use of NSAIDs at inappropriate doses and for extended periods can increase the likelihood of adverse effects. Some horses reportedly have a unique sensitivity to NSAIDs that may cause them to develop adverse effects at lower doses than for the general population of horses.

The study reported here was performed by use of horses concurrently included in a study conducted to examine kinematic gait analysis for the evaluation of lameness in horses receiving NSAID treatment. The purpose of the study was to evaluate effects of NSAIDs administered to horses in accordance with 2 protocols (PBZ or a combination of PBZ and flunixin meglumine at recommended dosages for short periods).

Materials and Methods

Animals—Twenty-four horses were used in the study reported here. Thirteen horses comprised the treatment group; these horses were concurrently enrolled in a study evaluating treatments for lameness. Eleven horses were included in a control group. The study was approved by the University of Missouri Animal Care and Use Committee.

The treatment group consisted of 12 geldings and 1 mare. Horses ranged from 6 to 22 years of age. Nine of the horses belonged to private owners; permission was obtained from each owner for use of the horse in the study. The control group consisted of 11 horses (4 geldings and 7 mares) that ranged from 3 to 26 years of age. Seven of the horses belonged to a university research and teaching herd, whereas the remaining 4 were client-owned horses that were in the hospital for reasons unrelated to this study or the concurrent study on evaluation of lameness; some of the horses were in the hospital for reasons that did not require medical treatment (eg, boarding a horse or a mare accompanying a foal). Permission for inclusion in the study was obtained for client-owned horses.

Procedure—Each horse was weighed and given a complete physical examination at time of admittance to the study. Horses were housed in the veterinary medical teaching hospital for 3 to 4 days for training and acclimation to the hospital environment. Each horse was fed their typical ration; all horses were fed 2 meals/d. Horses were exercised daily by hand walking or treadmill exercise (horses in the treatment group were assigned to the type of exercise on the basis of the schedule for the concurrent study on evaluation of lameness, whereas horses in the control group were assigned to receive exercise similar to that for the treatment group). Horses were moni-
Horses in the treatment group received NSAIDs in accordance with 2 treatment protocols by use of a crossover design. Horses were administered PBZ alone (2.2 mg/kg, PO, q 12 h, for 5 days) and a combination of PBZ and flunixin meglumine (PBZ, 2.2 mg/kg, PO, q 12 h, for 5 days; flunixin meglumine, 1.1 mg/kg, IV, q 12 h, for 5 days). There was a washout period of a minimum of 7 days between treatments; the order of treatment was randomly assigned. Client-owned horses were discharged to their owners during the washout period between treatments, whereas university-owned horses remained in the hospital facility.

On the first day of treatment (day 0), blood samples were collected from each horse. Blood samples were also obtained approximately 12 hours after administration of the last treatment on day 5. Samples were allowed to clot for 30 to 45 minutes; samples were then centrifuged and assessed for quality. Serum was decanted, and all serum samples (approx 4 mL/sample) were then frozen until subsequent analysis. At the end of the washout period, the procedures were repeated but each horse received the other treatment protocol.

Four randomly selected treated horses were evaluated by use of a 3-m gastroscope. The original design of the study was for all horses to undergo gastroscopy, but problems were encountered that precipitated use of the gastroscope during only portions of the study. Gastroscopy was performed before beginning each treatment (day 0) and repeated approximately 12 hours after administration of the treatment on day 5. Feed was withheld from horses for 12 hours before each gastroscopic procedure. Horses were sedated by administration of xylazine hydrochloride (100 to 150 mg, IV) to facilitate the gastroscopic procedure.

The cardia, greater and lesser curvatures, and margo pli-catus were evaluated for ulcers. Findings were recorded, and the mucosa was graded for ulcers. Ulcers were scored by use of the following scale: 0, intact epithelium with no appearance of hyperemia or hyperkeratosis; 1, intact mucosa with areas of reddening or hyperkeratosis; 2, small single or multifocal ulcers; 3, large single or multifocal lesions or extensive superficial ulcers; and 4, extensive lesions with areas of deep ulcers.

Control horses were subjected to similar exercise, feeding, and environmental influences as those for the treatment horses. Serum samples were obtained 2 to 3 days after admission and again 5 days later. Serum samples were treated in the same manner as for the treatment group and were stored frozen until subsequent analysis.

Each serum sample was thawed only once. A batch of samples was thawed and immediately submitted for analysis of total protein, albumin, and globulin concentrations by use of an automated chemical analyzer. Globulin concentrations were calculated by the chemical analyzer as the total protein concentration minus the albumin concentration.

Statistical analysis—A 3-factor, 2-way ANOVA was performed to analyze differences between serum total protein, albumin, and globulin concentrations with respect to treatments. Mean ± SD values were calculated. Inspection of raw data revealed that 2 horses in the treatment group had large decreases in serum total protein, albumin, and globulin concentrations for both NSAID treatments. Therefore, a second analysis was conducted that excluded data for these 2 horses and 2 randomly designated control horses. Any significant differences were evaluated by use of the Tukey pairwise multiple comparison procedure.

Results

One horse was euthanatized during the study because of complications associated with the combination treatment. This adult Thoroughbred mare was healthy on admission to the hospital. In the past, the horse had received PBZ as treatment for lameness and had not had visible adverse effects associated with PBZ administration. Gastroscopy performed on day 0 revealed no visible ulcers of the gastric mucosa of that horse. On day 0, the total protein concentration was 6.5 g/dL, albumin concentration was 3.7 g/dL, and concentration of globulins was 2.8 g/dL. On day 3 of the combination treatment, the horse had early clinical signs of ulcers, such as anorexia, lethargy, and peripheral and ventral edema.

By day 5 of the combination treatment, the horse had developed severe diarrhea. Gastroscopy performed on day 5 revealed extensive hyperkeratosis of the glandular mucosa with bile staining and large ulcerated areas in the glandular portion of the stomach. The nonglandular portion of the stomach had several ulcers (each of which was 1.5 cm in diameter) near the margo pli-catus. On day 5, total protein concentration was 4.0 g/dL, albumin concentration was 2.1 g/dL, and concentration of globulins was 1.9 g/dL. The horse was administered isotonic fluids to counteract dehydration from the severe diarrhea but continued to be anorectic and lethargic. On day 7, the horse had signs of colic (rolling, pawing, and thrashing intermittently) and was tachycardic (72 beats/min) and tachypneic (48 breaths/min). The decision was made to euthanatize the horse because of its deteriorating condition. Therefore, the horse was never administered PBZ alone, and data for this horse were not included in the statistical analysis.

On the basis of the Tukey pairwise multiple comparison procedure, results of the initial analysis that included data for 12 treatment and 11 control horses revealed a significant (P < 0.001) difference between control horses and horses administered the combination treatment, but not between the control horses and horses administered PBZ alone (P = 0.075) or between

![Figure 1](image-url)
As mentioned previously, 2 treatment horses had measured outcomes > 2 SD from the mean of the other horses in the treatment group for both treatments. Thus, data for these 2 treatment horses and 2 randomly selected control horses were excluded, and the analysis was repeated. The second analysis revealed significant differences between control horses and horses administered the combination treatment (P < 0.001), between control horses and horses administered PBZ alone (P = 0.049), and between horses administered the combination treatment and horses administered PBZ alone (P = 0.027; Figure 1).

Three of 4 horses examined by use of gastroscopy had no changes after administration of the PBZ treatment (grade 0/4), whereas the other horse had evidence of ulcers (grade 2/4) after administration of the PBZ treatment. For the same 4 horses, all had important findings and evidence of ulcers during gastroscopic examination conducted after administration of the combination treatment (2 had grade 2/4, 1 had grade 3/4, and 1 had grade 4/4). The horse with ulcers and a grade of 4/4 after administration of the combination treatment had ulcers and a grade of 2/4 after administration of PBZ alone (Figure 2).

**Discussion**

The purpose of the study reported here was not to comment on the effectiveness of combined NSAID treatment; rather, the intent was to look at the potential harmful effects associated with the concurrent administration of PBZ and flunixin meglumine versus use of PBZ alone. It appears that the combined use of these drugs is a common practice in equine veterinary medicine. Although both NSAIDs work through a similar mechanism of inhibition of COX expression of prostanoid production, individual NSAIDs may vary in their analgesic, anti-inflammatory, and antipyretic effects.1 Anecdotally, it is believed that use of a combination of NSAIDs may increase or maximize the benefits.

![Figure 2—Images obtained during gastroscopy of a representative horse before treatment (A), after administration of PBZ (2.2 mg/kg, PO, q 12 h, for 5 days; B), and after administration of a combination of PBZ and flunixin meglumine (PBZ, 2.2 mg/kg, PO, q 12 h, for 5 days; flunixin meglumine, 1.1 mg/kg, IV, q 12 h, for 5 days; C). There was a minimum washout period of 7 days between treatments. All images are of the lesser curvature of the stomach. In panel A, notice the normal gastric mucosa (pink areas). Panel B reveals mild damage of the gastric mucosa, with the green staining representing areas of hyperkeratosis. Panel C reveals extensive damage of the gastric mucosa, with the dark-red areas representing severe ulceration, green-white areas representing hyperkeratosis, and pink areas representing thinning mucosa.](Unauthenticated)
for each of the specific drugs. There is little information on the effects of combination treatments. It has been reported that administration of a combination of PBZ and flunixin meglumine does not change the pharmacokinetics of either drug. The potential for an increase in adverse effects has not been widely published.

The adverse effects of NSAIDs as a class of drugs have been determined. These include gastric and colonic ulcers, right dorsal colitis, impaction of the large colon, cecal impaction and rupture, and renal papillary necrosis. Some horses may also have increased sensitivity to NSAIDs and develop adverse effects more readily than most horses in the general population. A study of the in vitro effects of COX inhibitors suggests that PBZ and flunixin meglumine are more potent inhibitors of COX 1 than of COX 2. Cyclooxygenase 1 is considered the constitutive form of the enzyme and is responsible for several physiologic cell functions, such as mucosal cytoprotection, whereas COX 2 is an inducible form that is responsible for manifestations of inflammation. Both isoforms are expressed in the intestines of clinically normal horses. It is interesting that PBZ and flunixin meglumine are commonly used in horses and affect the protective constitutive COX to a greater degree than the inducible form of COX.

In the study reported here, total protein, albumin, and globulin concentrations were measured as indicators of potential adverse effects of the combination treatment. The reason for choosing these variables was the relative simplicity of sample collection. Hypoproteinemia and hypoalbuminemia are seen with changes in the integrity of the intestinal mucosa. Albumin is the most abundant protein in equine plasma and has a low molecular weight, which leads to a decrease in albumin in horses with inflammatory disease of the gastrointestinal tract. It is not typical that ulcers of the gastric mucosa alone would contribute to hypoproteinemia or hypoalbuminemia. The original design of the study called for gastroscopy of each horse, but difficulties with the gastroscope precluded that. Horses included in the study were given a thorough physical examination in an attempt to exclude horses with potential complicating problems.

Results of the initial statistical analysis provided evidence that a combination NSAID treatment significantly (P < 0.001) reduced serum total protein concentrations, compared with values for control horses. No significant (P = 0.073) difference was identified between the combination treatment and PBZ alone in the initial statistical analysis, and no difference was found between PBZ alone and the control horses. These results can be explained by a large variance in response to PBZ alone and the combination treatment in the treatment horses. It has been established that variation in sensitivity to NSAIDs exists among horses.

After inspection of the data, it was evident that 2 of the treatment horses had large responses to PBZ alone and the combination treatment, as measured by serum total protein concentrations. After calculating mean and SD values of the other treatment horses, it was determined that the 2 aforementioned horses had values > 2 SDs from the mean. On the basis of this evidence of potential outliers, data for these 2 horses (as well as data for 2 randomly selected control horses) were excluded from a second analysis. In the second analysis, there were significant differences between values for control horses and treated horses administered PBZ alone or the combination treatment as well as between treated horses when administered PBZ alone or the combination treatment. This is clear evidence that the combination treatment has a substantial negative effect on serum total protein concentration. In addition, the second analysis revealed that, depending on sensitivity of the specific horse, the combination treatment could be even more detrimental than treatment with PBZ alone.

Clinical signs of gastric ulcers in adult horses include a decrease in consumption of concentrates, postprandial episodes of colic, poor performance or failure to meet expectations, poor-quality coat, and decreased condition or failure to thrive. Eight of the 13 horses had clinical signs of gastric ulcers during administration of the combination treatment, including decreased consumption of concentrates, mild postprandial episodes of colic, and general mild lethargy. These signs were first evident in specific horses from days 2 to 4 of administration.

One horse had clinical signs when administered PBZ alone, although those signs did not appear until day 5 of treatment. This horse had been treated initially with the combination treatment and appeared to have milder clinical signs when administered the PBZ alone. The washout period for this horse between treatments was > 12 weeks because gastroscopy performed 4 weeks after completion of the combination treatment revealed that the horse still had gastric ulcers.

Necropsy of the horse that was euthanatized revealed gastritis, necrotizing typhlocolitis, and multifocal hepatitis, which were consistent with, but not pathognomonic for, toxic effects of NSAIDs. Attempts to isolate Clostridium difficile toxin yielded negative results, and serial cultures to isolate Salmonella organisms also yielded negative results.

Changes seen in total protein, albumin, and globulin concentrations were experimentally and clinically important in the horses during the combination treatment (Figure 1). Those horses on which gastroscopy was performed had marked ulcers after only 5 days of the combination treatment. Additionally, most horses in the study had clinical signs of NSAID toxicosis during administration of the combination treatment. Analysis of results of this study indicates a need for caution when considering use of combination NSAID treatment.

Future studies on this topic should include a larger pool of horses to counteract the influence of those horses that are extremely sensitive to any NSAID. In addition, studies that include histologic analysis of the right dorsal colon would be helpful in establishing the route of protein loss in these horses. Ultrasonographic evaluation of the right dorsal colon can be included in future studies. Evaluation of the kidneys should also be included.

The dosages used in the study reported here were reflective of those commonly used by equine practitioners. It would also be beneficial to study the use of PBZ at dosages of 2.2 mg/kg (the dosage used in this study) and...
4.4 mg/kg (the high end of the recommended dosage range for PBZ) and measure the effects on total protein, albumin, and globulin concentrations to determine whether there is a dose-dependent effect for PBZ.

References