Effectiveness of a unique dihydropyridine (BAY TG 1000) for prevention of laminitis in horses

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Objective—To determine whether a unique dihydropyridine (BAY TG 1000) would be beneficial in preventing laminitis in horses.

Animals—16 clinically normal adult horses.

Procedure—8 pairs of horses were used in a controlled double-blind study, using sex- and age-matched horses randomly assigned to treatment or control groups. Horses were subjected to carbohydrate overload to induce laminitis. Treated horses were administered BAY TG 1000 (30 mg/kg, PO, q 24 h) for 3 days. hoof wall surface temperature (HWST) and lameness were recorded at 4-hour intervals. The HWST was adjusted on the basis of time of onset of lameness and evaluated, using a repeated-measures ANOVA. Lameness 8 hours after onset and clinical status 72 hours after onset of lameness were evaluated, using Mann-Whitney procedures.

Results—Analysis revealed that BAY TG 1000 did not decrease the incidence of lameness but significantly ameliorated prodromal hypothermia, lessened the severity of lameness 8 hours after onset of lameness, and improved the clinical status of horses 72 hours after onset of lameness.

Conclusion and Clinical Relevance—Results support the conclusion that BAY TG 1000 was protective when used in prevention of laminitis. The drug decreased severity and improved clinical status (recovery) of induced lameness, which was interpreted to mean that the drug’s actions were on mechanisms important but secondary to primary causal mechanisms of laminitis. Therefore, drugs that enhance digital perfusion via alteration of rheologic activity may have potential use in the prevention and management of laminitis in horses. (Am J Vet Res 2002;63:443–447)

In horses with laminitis, there is increasing evidence that damage to the submural laminar interface exists prior to the initial onset of lameness. Analysis of data reveals that decreased digital perfusion exists during the prodromal phase of laminitis induced by carbohydrate overload or administration of black walnut extracts. With the carbohydrate-overload model, prodromal hypoperfusion appears to last approximately 12 hours and then becomes undetectable prior to onset of lameness.

Coagulative dysfunction, formation of platelet thrombi in the laminar microcirculation, and an increase in expression of cyclooxygenase enzyme systems by digital endothelial and vascular smooth muscle cells during the prodromal phase all support the concept that digital lesions are developing prior to the onset of lameness. A histologic study has revealed obvious disease during the phase immediately after the onset of lameness; those results have been interpreted as evidence of progression of prodromal lesions following onset of lameness.

Two general hypotheses have been proposed for the causal mechanism of laminitis. The vascular hypothesis proposes that ischemia of the submural laminar interface is the initial or primary lesion leading to the onset of clinical disease. Potential mechanisms responsible for the ischemia include vasoconstriction of the digital vasculature, arteriovenous shunting below the level of the laminar interface, vascular compression secondary to compartment injury, or countercurrent shunting of oxygen in the extended microcirculation of the lamina. In the vascular hypothesis, platelet activation and inflammatory events are seen as pathophysiologic responses to the ischemic insult.

The second general hypothesis, the toxic-metabolic hypothesis, contends that a laminitis-triggering factor of enteric origin is the initial etiologic agent of the disease. Hypothetically, lactic acid, endotoxin, enterotoxin, tumor necrosis factor, and Streptococcus bovis exotoxin have all been incriminated as laminitis-triggering factors. It has been proposed that these factors are absorbed from damaged bowel or arise secondary to absorbed agents. These agents, or mediators produced by them, circulate to the hooves where they initiate platelet activation, damage or alter epidermal cells, or induce inflammation. These changes culminate in epidermal damage that includes loss of the attachment of the dermal-epidermal basement membrane and mechanical collapse of the foot. In this hypothesis, the vascular changes are secondary to the thrombotic-embolic event, inflammatory process, or trauma as the foot fails mechanically.

Regardless of which 1 of these hypotheses is valid, the prophylactic use of agents that promote digital perfusion via enhancing RBC deformability or decreasing cell-to-cell adhesion (platelet or leukocytic) should positively affect the clinical course of laminitis. A unique dihydropyridine, BAY TG 1000, has pronounced rheologic but little vasodilatory activity.
the study reported here, we tested the hypothesis that BAY TG 1000 would prevent laminitis induced by carbohydrate overload in horses.

**Materials and Methods**

**Animals**—Sixteen clinically normal adult horses were used in the study. All horses had healthy feet and were included when they met the following criteria: did not have a history of recent lameness, did not have evidence of clinical lameness, and did not have radiographic evidence of disease on lateromedial and dorsopalmar radiographs of the front feet. After entrance into the study, horses were matched as pairs on the basis of sex and age, and 1 of each pair (ie, 8 horses) was randomly assigned to receive a control treatment (the carrier for the active ingredient), whereas the other 8 horses were administered BAY TG 1000. Both treatments were prepared as pastes and encoded by the manufacturer to ensure that investigators were unaware of the treatment administered to each horse.

Each pair of horses (control and treatment) was placed in a controlled environment (mean ± SD, 19 ± 1 C; 70% humidity). Horses were fitted with instruments for acquisition of hoof wall surface temperature (HWST), after which they were allowed 24 hours to accommodate to the laboratory environment. Skin surface thermistors were placed on the dorsal surface of the hoof wall at a point approximately a third of the distance between the coronet and the ground-contact surface. This location approximates the middle of the dorsal partial surface of the distal phalanx. Thermistors were insulated from the environment, using 2 X 2-in square styrofoam pads secured with elastic tape. All thermistors used in this study were calibrated prior to use to ensure a common baseline. The HWST recording system was capable of differentiating hoof wall temperature to ± 0.05 C. Thermistor leads were secured to the forelimbs, using light bandages.

**Procedures**—The study was conducted as a concurrent controlled double-blind trial. The experimental protocol was reviewed and approved by the Laboratory Animal Care Committee at Texas A&M University.

During a 24-hour baseline period, horses were evaluated for behavior and recording of HWST at 4-hour intervals. At each 4-hour evaluation, a subjective assessment was completed of the willingness of each horse to walk from a stall that was deep filled with shavings onto a floor covered with a firm rubber mat and then onto a concrete floor. Each horse then was restrained for 5 to 6 minutes in a standing position on a floor covered with a firm rubber mat, and it was exercised on a floor covered with a firm rubber mat and then onto a concrete floor. Each horse then was restrained for 5 to 6 minutes in a standing position on a floor covered with a firm rubber mat, and then onto a concrete floor. Each horse then was restrained for 5 to 6 minutes in a standing position on a floor covered with a firm rubber mat. Thermistor leading was secured to the forelimbs, using light bandages. Following the 24-hour baseline period, horses were fed a high-carbohydrate meal to induce laminitis. Four hours after administration of the meal, treatments were initiated. Treatment horses were administered BAY TG 1000 (30 mg/kg, PO), and control horses were orally administered an equal volume of the carrier substance. Treatments were administered 4, 24, and 48 hours after administration of the high-carbohydrate meal. Syringses containing control treatments or BAY TG 1000 were encoded so that the investigators were unaware of the treatment given to each horse. The person responsible for clinical evaluation was not present when the horses were treated. The dose of BAY TG 1000 used in this study was determined on the basis of results of experiments that established effective circulating concentrations in horses.

During the 72-hour period after the high-carbohydrate meal was fed, each pair of horses was examined at 4-hour intervals to determine severity of systemic disease, including diarrhea, pyrexia, and colic, that typically accompanies feeding a carbohydrate overload. The HWST and rectal temperature of each horse also was recorded, and subjective evaluation of lameness was conducted, using the same protocol as during the baseline period. Time of onset of lameness (time 0) was defined as the time point at which a horse appeared reluctant to come out of the stall, had a change in gait, or had stiffness when turning. When it was questionable whether a horse had onset of lameness at a specific time point, that horse was reassessed 4 hours later; an increase in signs of lameness at this subsequent time point was used to confirm that the preceding time point should be designated as the time of onset of lameness.

Horses were evaluated twice during the study to determine the clinical response to treatment. Eight hours after the onset of lameness, severity of lameness was subjectively defined, using the Obel grading system. Also, clinical status of horses was assessed 72 hours after the onset. At that time, horses were categorized as recovered (lameness was not evident as the horse walked and made turns on a hard surface), mildly lame (results of aforementioned lameness examination were still positive), or severely lame (horse was required to be euthanatized because of the severity of lameness). At the conclusion of this study, all horses were administered appropriate analgesic treatment if the lameness was mild or euthanatized if the lameness exceeded an Obel grade of 3 at the examination conducted 72 hours after onset of lameness.

**Statistical analysis**—Only data for horses that became systemically ill (ie, developed diarrhea and pyrexia secondary to administration of the high-carbohydrate meal) were included in statistical analysis. Whether a horse received BAY TG 1000 or control treatment was revealed after data collection was completed.

The HWST for each horse was adjusted to the time of onset of lameness (time 0), and values for HWST were plotted against time. Data were compared over time, using a repeated-measures ANOVA with time and treatment as factors and HWST as the dependent variable. An unpaired t-test was used to confirm that HWST differed significantly between groups at the baseline period, 12 hours prior to onset of lameness (prodromal period), at onset of lameness, and 8 hours after onset of lameness. Finally, a paired t-test was used to confirm whether baseline HWST within each group differed significantly from HWST obtained for the prodromal period, at onset of lameness, and 8 hours after onset of lameness.

Frequency distribution of categoric data (Obel scores) determined 8 hours after onset of lameness and clinical status determined 72 hours after onset of lameness for the control and treatment groups was calculated and compared, using Mann-Whitney analysis. A value of P ≤ 0.05 was used to define significant differences.

**Results**

Horses used in this study consisted of 12 Quarter Horses or Quarter Horse crosses, 2 Appaloosas, and 2 Arabians. Twelve horses were geldings, and 4 were mares. Mean ± SD age was 7.8 ± 2.3 years, and mean body weight was 352 ± 44.3 kg.

Twelve of 16 horses had diarrhea or pyrexia typical of horses used in the carbohydrate-overload model. Two horses in the treatment group and 2 control horses did not have systemic illness or lameness. (Table 1). Of the 12 horses that had systemic signs associated with carbohydrate overload, all developed lameness consistent with acute laminitis. Characteristics of
lameness in these horses were consistent with that of our previous experience with the use of the carbohydrate-overload model. Specifically, the initial lameness was subtle, consisting of a stiffness of gait and reluctance to step onto hard surfaces.\footnote{Mean time of onset of lameness did not differ significantly (P = 0.919) between the control (35.33 ± 8.5 hours) and treatment (36.00 ± 13.0 hours) groups. Typical of horses subject to carbohydrate-induced lameness, the lameness was most severe at 8 hours after onset and began to decrease over time. In 1 horse, lameness persisted (Obel grade of 3) for 72 hours after onset, and that horse was euthanatized.}

Control horses had digital hypothermia during the period 8 to 12 hours prior to the onset of lameness and had slight digital hyperthermia following onset of lameness (Fig 1). In horses treated with BAY TG 1000, prodromal hypothermia was not evident, and hyperthermia after the onset of lameness was pronounced. Results of repeated-measures ANOVA indicated that 12 hours prior to onset of lameness, the HWST of the control horses was significantly (P = 0.047) lower than at all other times and that differences in HWST were not detected at any time point (P = 0.445) in the horses treated with BAY TG 1000.

The HWST in the control group during the base-

Discussion

The incidence of laminitis in the control group was typical of that observed when the carbohydrate-overload model is used. Two horses in each group did not develop diarrhea or pyrexia following carbohydrate overload, but that is not unusual and is attributed to biological variation among subjects. The justification for excluding data for these horses from the analysis was based on the premise that systemic changes are necessary to initiate digital mechanisms that induce laminitis. Therefore, inclusion of data for those horses would have been illegitimate.

Use of HWST as a valid indicator of digital perfusion has been reported.\footnote{Baseline HWST for the treatment horses was 32.7 ± 1.2 C, which was not significantly (P = 0.941) different from that of the control group. At the prodromal point 12 hours before onset of lameness, HWST was 32.0 ± 2.7 C, and at the onset of lameness, HWST was 32.8 ± 1.9 C. Neither of these values were significantly different from the baseline value (P = 0.676 and 0.891, respectively). At 8 hours after onset of lameness, HWST for the treatment horses was 33.8 ± 0.9 C and was significantly (P = 0.014) increased, compared with baseline HWST. The HWST during the prodromal period, at the onset of lameness, and at the time points after onset of lameness were not significantly different between groups, as determined by results of an unpaired t-test.

Severity of lameness 8 hours after onset of lameness indicated an effect attributable to treatment (Table 1). In control horses, median score was Obel grade 3 (1 horse had Obel grade 1, 1 had Obel grade 2, and 4 had Obel grade 3). Median Obel grade in treatment horses was 1.5 (3 horses had Obel grade 1, 2 had Obel grade 2, and 1 had Obel grade 3). Mann-Whitney analysis indicated that the Obel grades of the control horses were higher, but not significantly so (P = 0.066), than those of the treatment horses.

Seventy-two hours after onset of lameness, 4 of 6 control horses were still mildly lame, 1 was severely lame and was euthanatized because of severity of lameness, and 1 had recovered. In the treatment group, 4 of 6 had fully recovered, and 2 were still mildly lame. Mann-Whitney analysis indicated that the clinical status of the treatment horses was better, but not significantly so (P = 0.066), than that of the control horses.
stopped collecting HWST data 8 hours prior to onset of lameness. Horses treated with BAY TG 1000 did not have prodromal hypothermia, except for 1 horse that had pronounced hypothermia. Hyperthermia after onset of lameness was detected in treatment horses. If hypothermia and hyperthermia reflect changes in submural blood flow, analysis of these data infers that treatment with BAY TG 1000 maintained perfusion during the prodromal and acute phases of laminitis.

Detection of lameness during the early stages of laminitis induced by carbohydrate overload cannot be scored by use of the classic Obel grading system. With this model, initial lameness typically is subtle and becomes more severe during 12 to 24 hours after onset.\(^{23}\) The rate at which lameness becomes more severe varies markedly among horses. Thus, the lowest Obel grade (grade 1), which is characterized by a horse incessantly lifting its forefeet when standing, not having obvious lameness when walking, and having a stilted gait when trotting, may not be reached until the horse has been lame for 4 to 8 hours. Therefore, the protocol described here for detecting the initial onset of lameness was developed and used consistently throughout the study.

Horses treated with BAY TG 1000 in the study reported here had amelioration of prodromal hypothermia, decreased severity of lameness at 8 hours after onset of lameness, and improved recovery at 72 hours after onset of lameness. The Obel grade at 8 hours and clinical status at 72 hours after onset did not reveal significant effects of the drug. This may relate to the relative coarseness of the subjective scoring systems (Obel and clinical status) used. From these results, we concluded that BAY TG 1000 had a beneficial effect, but because the incidence of lameness in both groups was equal, the drug, as used in this study, did not prevent laminitis.

Interpretation of these results included the fact that efficacy of BAY TG 1000 was limited, because its active between the insult and initial onset of lameness. Alternatively, these results can be interpreted to mean that the drug was acting on primary mechanisms of laminitis, but the dose used in this study was insufficient to prevent laminitis.

Given the actions of BAY TG 1000, results of this study can be interpreted to indicate that a primary vascular pathophysiologic process is an inciting mechanism of laminitis. If digital submural perfusion is decreased during prodromal laminitis to a point that the tissues become ischemic, it follows that an agent that enhances microcirculatory flow should decrease severity of that ischemia and lessen severity of the disease. Agents that decrease viscosity of blood at the microcirculatory level would allow increased perfusion by increasing the ability of the blood to transit the microcirculation.

It is documented that ischemia elicits a cascade of proinflammatory factors including tumor necrosis factor, prostanooids, endothelins, and selectins, the latter of which increase leukocytic adhesion to the microcirculatory endothelium, affect platelet dysfunction, and decrease erythrocytic deformability.\(^{26-31}\) Together, these further disrupt the microcirculation secondary to the ischemic insult. Logically, if the primary mechanism of laminitis involves ischemia, the preventive use of agents that disrupt this cascade should be beneficial.

These same data can also be used to support versions of the hypothesis for a laminitis-triggering factor that proposes that some agent derived or absorbed from the large bowel is acting to initiate intravascular aggregation of leukocytes, platelets, or erythrocytes; induce an inflammatory response in the digital circulation; or directly affect viability of the laminar epithelium resulting in release of proinflammatory cytokines. In each of these instances, activity of an agent that combats cellular aggregation and enhances rheologic properties of erythrocytes should be beneficial.

The study reported here provides information to give us a better understanding of the pathophysiologic processes for developmental and acute laminitis. The clinical importance of this study lies in the conclusion that drugs such as BAY TG 1000 as well as pentoxifylline, other methylxanthines, or other dihydropyridines may have a potential place in the management of developmental and acute laminitis. The relative effectiveness and effective doses of these agents have yet to be defined.


References


