Effectiveness of glyceryl trinitrate for enhancing digital submural perfusion in horses

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Objective—To evaluate the clinical efficacy of topically administered glyceryl trinitrate (GTN) for inducing digital submural vasodilation in clinically normal horses.

Animals—7 adult horses without foot abnormalities.

Procedures—A concurrent-control crossover design was used to determine whether topical application of GTN ointment for prevention or treatment of laminitis would result in a detectable increase in digital perfusion. Heat-acclimated horses instumented for detection of wall surface temperature (HWST), mean systemic pressure, and heart rate were used. Horses were exposed to cold to induce digital vasoconstriction and treated with GTN in an attempt to induce digital vasodilation.

Results—Application of GTN failed to induce an increase in digital submural perfusion but did induce a mild decrease in mean systemic pressure.

Conclusions and Clinical Relevance—Topical application of 60 mg of GTN as a 2% ointment on the skin over the major vasculature in the region of the proximal interphalangeal joint (pastern) of horses was not effective in significantly increasing digital perfusion. A decrease in mean systemic pressure following treatment was observed, implying that the drug was absorbed. Use of GTN may result in a decrease in digital submural perfusion secondary to induction of peripheral constriction or a decrease in digital perfusion pressure. (*Am J Vet Res* 2002;63:648–652)

Controversy exists regarding whether digital perfusion is increased, decreased, or unaffected in horses with laminitis.[1-3] It has been hypothesized that some of the discrepancies regarding digital hemodynamics can be explained by evaluating the interpretations and conclusions from other studies on the basis of phase of the disease.[3-6,a,b] During the developmental phase, there is mounting evidence that a prolonged significant decrease in digital vascular perfusion exists. Specifically, this evidence consists of digital hypothermia in the period 8 to 12 hours prior to the onset of lameness,[3-5] laminar histopathologic changes that are consistent in distribution and character with an ischemic event,[a] and the reduced incidence and severity and enhanced recovery that are achieved through the use of agents that enhance digital perfusion when used as preventives.[6,b]

Data also support the fact that as lameness appears, signaling the onset of the acute phase of laminitis, submural digital perfusion is increasing but is not substantially higher until lameness has been evident for 8 to 12 hours.[1,3] If an ischemic process is involved in the developmental phase, it follows that the increase in perfusion during the acute phase is consistent with reperfusion hyperemia. As the disease progresses temporally, it has been postulated that reperfusion injury, inflammation, compartment injury, traumatic tearing of vessels, and altered vascular reactivity contribute to a secondary decrease in perfusion during the late acute, subacute, and chronic phases of the disease.[1]

The potential causes for a decrease in submural perfusion during the developmental phase of laminitis have been poorly explored. Various studies have provided physiologic, histologic, or histopathologic evidence for the working hypotheses that the decrease in submural perfusion is attributable to compartment injury,[7] inappropriate stimulation or response of digital arteriovenous shunts,[8] vasospasm,[4,7] coagulopathies,[9,10] and a pathologic countercurrent oxygen mechanism.[3,4] However, critical experiments necessary to validate these possibilities as causes or secondary mechanisms in developmental laminitis are lacking.

The mechanistic hypotheses based on this evidence indicate that pharmacologic agents that enhance digital perfusion may be an effective means of limiting the disease during the developmental phase and may be used as a means of treatment during the late acute, subacute, and chronic phases.[11] Understanding of the vascular control in the feet of horses, which necessarily serves as the basis of pharmacologic manipulation, is somewhat limited, and assumptions frequently are made on the basis of cutaneous circulation in other species. Given the architectural complexity of the digital circulation in the feet of horses, the validity of these assumptions is difficult to predict.

Several agents have been advocated for use and partially tested for their clinical efficacy as preventatives during the developmental phase of laminitis. Evidence suggests that the use of α-adrenergic receptor antagonists,[b] high doses of heparin,[c] and dihydropyridines[6] provide some degree of protection when used as preventatives during the developmental phase of laminitis. More recently, nitrous oxide has been offered as another agent for management of horses with laminitis. This latter application is predicated on the fact that equine arterial endothelial cells are capable of producing nitric oxide,[12,13] systemic infusion of L-arginine enhances digital perfusion in horses sedated with detomidine

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hydrochloride and butorphanol tartrate, and transdermal application of **glyceryl trinitrate (GTN)** in horses with chronic laminitis is efficacious, as determined on the basis of a subjective improvement in lameness.[14,15]

On the basis of those studies and their interpretations, transdermal application of GTN as a 2% ointment has become a popular therapeutic regimen for horses with developmental and acute laminitis. Typically, GTN is applied over the major vasculature on the pastern or coronary band. Intuitively, this is intended to potentiate local digital effects while limiting potential hypotensive systemic effects. However, there is a paucity of data that could be used to validate whether sufficient absorption at this site would result in the desired therapeutic effect. We chose to investigate whether GTN applied topically (ie, consistent with clinical use) was capable of inducing significant enhancement of submural perfusion and whether it was accompanied by a systemic hypotensive effect. The hypothesis tested was that GTN applied over the palmar digital vessels of the pastern would be effective in producing digital vasodilation without development of systemic hypotension.

Materials and Methods

The study was conducted as a concurrent control, crossover design, using GTN (2%) and a control treatment (aqueous gel that did not contain GTN). All protocols used in this study were approved by a university laboratory animal care committee.

Seven horses with normal feet were used in the study, consisting of 4 Arabians, 1 Quarter Horse, 1 Paint, and 1 Appaloosa. There were 4 geldings and 3 mares. Horses ranged from 4 to 9 years of age (mean, 6.2 years), and mean ± SD body weight was 397 ± 45 kg. Inclusion criteria for horses used in the study were that the horses did not have a history of recent lameness, did not have evidence of clinical lameness or laminitis, and did not have radiographic evidence of disease on lateromedial and dorsopalmar radiographs of the forefeet.

Horses were acclimated to heat and then subjected to cold challenge-exposure during the experimental period. Heat acclimation was accomplished by maintaining the horses on pasture for 3 months during a period when mean ambient temperature and humidity were 28.7 ± 1.54 C and 66.86 ± 10.58%, respectively. Cold challenge-exposure was achieved by completing the experiments in a controlled laboratory environment in which ambient temperature and humidity were maintained at 15.0 ± 1.8 C and 78%, respectively.

Horses were weighed when the laboratory conditions were initiated. Horses were maintained overnight in the laboratory to allow temperature of the feet to stabilize. One hour before initiation of each experiment, the pastern regions of both forfeet were shaved, and the hooves were instrumented to enable detection of **hoof wall surface temperature (HWST)**. Skin-surface thermistors[d] were placed on the dorsal surface of the hoof wall at a point approximately one third of the distance between the coronet and the ground surface. This location approximates the middle of the dorsal parietal surface of the distal phalanx. Thermistors were isolated from the environment, using Styrofoam pads (2 × 2-in squares) secured with elastic tape. All thermistors had been previously calibrated to ensure a common baseline. The HWST recording system was capable of differentiating temperatures within ± 0.05 C. Thermistor leads were secured to the forelimbs, using light bandages.

Heart rate and systemic blood pressure were monitored to determine systemic effects. Mean systemic blood pressure and heart rate were measured noninvasively, using a tail-cuff pressure manometer.[e] The tail cuff was placed on each horse 1 hour before the initial measurement and was secured to the horse throughout the experimental period. Horses were not tranquilized or administered sedatives.

Subsequent to instrumentation, each horse was subjected to a 1-hour control period during which HWST, mean systemic pressure, and heart rate were recorded at 15-minute intervals. Following the control period, both forefeet were treated with GTN ointment or the carrier gel applied to the skin over the medial and lateral palmar digital vessels in the pastern regions. Treatment (GTN [2%]) or control gel was applied as two 2.54-cm aliquots on each forelimb (total dose for each horse, 10.16 cm). Total dose of GTN applied was 60 mg (approx 0.15 mg/kg). Following application of GTN or control gel, the pastern area was covered with a plastic wrap and a light bandage.

Following application of GTN or control gel, HWST, blood pressure, and heart rate were recorded at 15-minute intervals for 4 hours. At the end of the data collection period, bandages were removed, and the limbs were washed to remove any remaining GTN or gel. Horses then were returned to outside paddocks. Horses were allowed at least 24 hours before being subjected to the alternate treatment, using the same protocol.

Statistical analysis—Mean HWST of both forefeet was determined and used in all calculations. Mean ± SD was calculated for HWST, blood pressure, and heart rate at each sample collection point and examined for distribution and variance. Means for the measurement variables were compared between the 2 treatment groups during the control period, using an unpaired *t*-test to ensure that the groups were not significantly different. Data were adjusted to the time of initiation of treatments and examined. Subsequently, data were compared over time by use of a repeated-measures ANOVA, with treatment, time, and horse as independent factors. When significant differences were detected, a comparison of means was completed, using the least-significant difference test. A value of $P \leq 0.05$ was used to describe significance.

Results

During the control periods, 5 of 7 heat-acclimated horses used in this study had HWST that was approximately equal in both forefeet (mean ± SD, 19.13 ± 0.38 C). One horse had a mean HWST of 29.92 ± 0.2 C and 30.02 ± 0.3 C on both days of treatment for the left and right forefeet, respectively. In addition, 1 horse had a mean HWST of 19.32 ± 0.1 C for the forefeet on the day of the GTN treatment, but on the day of application of the control gel, mean of the left forefoot was 19.4 ± 0.2 C whereas that of the right forefoot was 30.03 ± 2.4 C. None of the horses had signs of discomfort or aversion to handling during the course of the study.

During the control periods, mean HWST of horses for application of the control gel was 21.74 ± 5.2 C, and mean HWST for application of GTN was 20.62 ± 3.9 C; these values did not differ significantly ($P = 0.385$). Following treatment with the control gel or GTN, mean HWST was 22.52 ± 4.9 and 20.71 ± 4.2 C, respectively. We did not detect evidence of an altered HWST for either treatment during the course of the study (**Fig 1**). Use of repeated-measures ANOVA indicated that GTN treatment did not significantly ($P = 0.397$) affect HWST, compared with values for the control gel.

Heart rates of horses during the control period did not differ significantly ($P = 0.418$) for horses prior to administration of control gel or GTN (47.3 ± 10 and 43.3 ± 8 beats/min). Following application of treatment, means for the GTN and control gel were 41.8 ± 7 and 40.9 ± 7 beats/min, respectively. Although it appeared visually that GTN treatment increased heart rate in specific horses, results of repeated-measures ANOVA confirmed that heart rates did not differ significantly ($P = 0.157$) between treatments.

Mean blood pressure of horses during the control period prior to administration of GTN (92.0 ± 17 mm Hg) was slightly increased relative to that of the horses prior to administration of the control gel (86.8 ± 15 mm Hg); however, these values did not differ significantly ($P = 0.067$). Mean systemic pressure of horses after application of GTN was 83.4 ± 14 mm Hg, whereas mean systemic pressure after application of the control gel was 89.5 ± 17 mm Hg (**Fig 2**). Results of repeated-measures ANOVA indicated that a time-by-treatment effect was evident ($P = 0.015$). Separation of means for time and treatment did not reveal significant differences among time periods or between treatments.

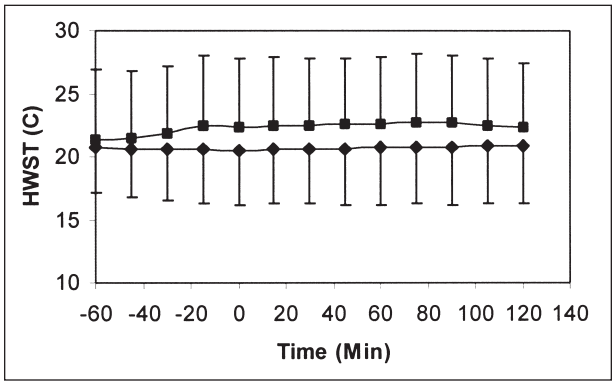

Figure 1—Mean ± SD hoof wall surface temperature (HWST) of 7 horses after application of glyceryl trinitrate (GTN; diamonds) and control gel (squares). Data were adjusted to time of treatment application (time 0). Mean values for HWST before or after application of GTN and control gel were not significantly ($P \leq 0.05$) different.

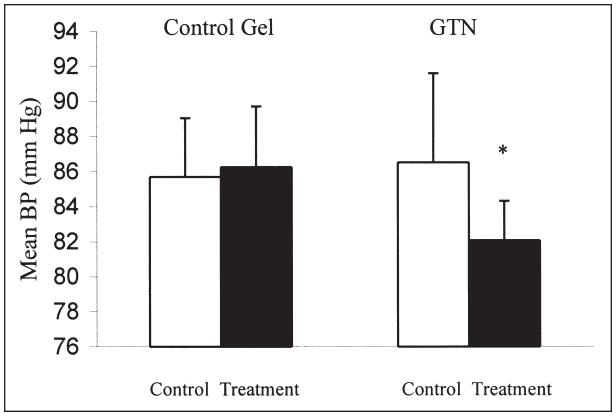

Figure 2—Mean ± SD systemic blood pressure (BP) during the period before application (control period; open bars) and after application (treatment period; solid bars) of control gel and GTN in 7 horses. *Mean pressure recorded during the treatment period was significantly ($P \leq 0.05$) different from that of the control period.

Discussion

The cold challenge-exposure imposed on heat-acclimated horses in the study reported here was designed to induce protracted vasoconstriction of the digital circulation to optimize our ability to detect a vasodilatory response induced by the GTN. In another study,[3] it was indicated that the exposure of heat-acclimated horses to sustained cold ambient temperatures induces digital vasoconstriction. The mechanism for the digital hypothermia reflects an attempt to maintain thermostasis via shunting of blood away from cutaneous beds, including those of the feet. It is unknown whether the mechanisms responsible for vasoconstriction are mediated by local or central input.

Spontaneous vasoregulatory escape periodically is evident during these conditions.[3] Vasoregulatory escape is a common physiologic vascular phenomenon and is attributed to local build up of metabolic products or oxygen debt associated with insufficient blood flow through tissue.[3] Detection of this phenomenon supports the concept that digital vasoconstriction induced by cold ambient temperatures may involve central input. The increased HWST seen during episodes of vasoregulatory escape further validates the fact that acute changes in digital perfusion can be readily detected by use of HWST. In the study reported here, unilateral increase of HWST seen in 1 horse was attributed to this escape phenomenon. The increased HWST seen during episodes of vasoregulatory escape validates the fact that increases in digital perfusion can be readily detected by use of HWST as an index of perfusion.

The ability of digits of horses to undergo prolonged periods of reduced perfusion during these experimental conditions was attributed to a low metabolic rate of the digital cutaneous structures. Although the metabolic rate of digital dermal and epidermal tissues is unknown, we assumed it was low. The HWST represents the thermal equilibrium between the ambient temperature to which a foot is exposed and internal exothermic heat sources. Thus, the conditions imposed on a foot by the experimental procedure we used here intuitively increased the duration during which the digital cutaneous structures could be expected to withstand decreased perfusion. Logically, the reduced ambient temperature further decreased the metabolic rate of the submural tissues and thereby delayed metabolic-induced vasodilation.

The lack of vasoconstriction seen in 1 horse was attributed to biological variation. The possibility that the sustained HWST seen in this horse was the result of thermoregulatory escape is reduced by the fact that the HWST was in both forefeet on both days in which the horse was used. Other than the subjective impression that this horse appeared more anxious or nervous than the other horses used, we did not have evidence that this horse differed from the other horses. Thus, the data from this horse were included in the analysis.

The lack of change in HWST recorded in this study is consistent with the interpretation that GTN applied to the feet of horses has little effect in enhancing digital perfusion of clinically normal horses in which vasoconstriction is evident prior to application.

Lack of change in the HWST was not attributable to the inability of the HWST technique to detect increased digital perfusion, as indicated by the ability of the technique to detect the aforementioned vasodilatory escape. The lack of response seen in this study also cannot be explained by a lack of sufficient absorption of GTN. A recorded decrease in mean systemic blood pressure and a nonsignificant increase in heart rate following application of GTN is evidence that the GTN was absorbed. Logically, the observed decrease in systemic blood pressure was the consequence of a decrease in vascular resistance, indicating that there was substantial absorption.

A systemic response despite the lack of a digital response can be rationalized by hypothesizing that the principal route of absorption of the GTN was via local cutaneous microcirculation and lymphatics. Because flow through these vessels is directed proximally toward the central circulation, little of the absorbed GTN would have gained access to the digital submural circulation. The only enhancement of digital perfusion subsequent to local application would logically have to result from a direct effect on the underlying arteries or from GTN that recirculated to the digital area via the systemic circulation.

Arteries and veins of the digital submural circulation located distal to the site of application of GTN are characterized by thickly muscled walls associated with small lumens.[4] This allows the possibility that the mechanisms responsible for the thermal-induced decreased perfusion included constriction of vessels within the hoof in addition to the arteries proximal to the hoof. The effect of any GTN-induced relaxation of the arteries proximal to the hoof and proximal to the constricted submural circulation would minimize any effect on digital perfusion.

Assuming that most of the GTN was absorbed via the local microcirculation and lymphatics and was carried systemically, 2 mechanisms may explain the decreased likelihood of producing an effect on the digital microcirculation. First, the dose used in this study was calculated at a rate of 0.15 mg/kg, which was applied to a small surface area of relatively thick epidermis. In humans, a dose for a 70-kg person is calculated at a rate of 0.43 to 0.86 mg/kg applied over an 8-cm^2 area of thin truncal skin to achieve coronary artery dilatation.[16,17] For the conditions in the study reported here, it is logical to assume a lower rate of absorption and a substantial increased dilution in our horses. Coupled with the relatively short half-life of GTN,[18] recirculation to the foot in quantities sufficient to have an effect may be unlikely.

The second factor that potentially may have contributed to the lack of an observed vasodilatory response in the digits could have included physiologic accommodation of the vasodilatory effects of GTN. This accommodation includes the development of resistance to the continuous application of GTN.[19] Although such resistance in other species is not typically seen until the duration of continuous application exceeds 12 hours, the phenomenon has not been characterized in horses and may be evident sooner.

On the basis of these results, it is possible to hypothesize that when GTN is used as described, there is a decrease in digital perfusion. One mechanism for this decrease is via the homeostatic reflexes responsible for maintaining systemic blood pressure. In this hypothesis, GTN-induced systemic hypotension evokes constriction of the digital circulation in an attempt to maintain systemic blood pressure. Intuitively, such an increased amount of stimulation could mask any vasodilatory effects of GTN in the digits, especially if the quantity of GTN was low.

A second physiologic mechanism by which GTN could cause an additional adverse effect is that the GTN was reaching the digits in sufficient quantities to induce a vasodilatory effect, but because of the drug-induced systemic hypotension, the digital flow rate was sufficiently reduced to cause digital hypoperfusion. This second mechanism is further complicated by the unique architectural complexity of the submural microcirculation. The submural microcirculation is extensive, with arterial and venous components arranged in close proximity. This establishes the anatomic conditions for a countercurrent flow phenomenon similar to that described for the intestinal microvilli.[20] Hypothetically, in the feet of horses, a substantial reduction in systemic inflow pressures secondary to GTN activity on systemic resistance vessels could lead to decreased digital perfusion pressure that, in turn, induces the countercurrent shunting of nutrients, metabolic waste, and heat.

As indicated previously, the experimental conditions used were chosen to enhance our ability to detect a vasodilatory response. These experimental conditions interfere with the ability to detect a reduction in digital perfusion.[3] Simply, it is difficult to detect a decrease in HWST when the vessels in a foot are already constricted. Thus, interpretation of the data from the study reported here can only be applied to the question of whether the use of GTN enhances digital perfusion. Determination of whether submural perfusion was decreased following GTN treatment, using HWST as an indicator of perfusion, would require performing this experiment in cold-acclimated heat-challenged horses.

The implication of these data in regard to the use of GTN in horses with developmental laminitis is unclear. At best, the use of GTN as described here can be seen as having little if any effect. Alternatively, assuming GTN compromised digital perfusion via reflex constriction of the digital circulation or secondary to a reduced digital flow with or without countercurrent shunting, its use is contraindicated. In the early-acute phase of laminitis, a time when hemodynamic data indicate that digital flow is enhanced secondary to reperfusion hyperemia, there is little foreseeable benefit in the use of GTN.

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