

PUTTING OUT THE FIRE - LAMINITIS PREVENTION

Professor Christopher C. Pollitt. BVSc, PhD.

Australian Equine Laminitis Research Unit

School of Veterinary Science

Faculty of Natural Resources, Agriculture and Veterinary Science

The University of Queensland

Qld 4072 AUSTRALIA

c.pollitt@uq.edu.au

www.laminitisreserach.org

Introduction

From the outset it must be stated that a therapeutic regime, using biological or chemotherapeutic agents, able to arrest or block the triggering of laminitis, does not exist. On the other hand, there is a plethora of remedies, used empirically, that symptomatically help the horse after it has acquired laminitis. It is more the extent and severity of the lamellar pathology that influences the outcome for the horse, not the treatment regimen itself. An effective laminitis preventive may emerge when the mechanism behind the disintegration of the anatomy of the hoof wall lamellae is fully understood. Our discovery that a class of enzymes appears to be involved in the lamellar failure of laminitis has led us to commence trials of proteinase inhibitor therapy, specifically targeted at hoof wall matrix metalloproteinases.

Since laminitis usually develops as a sequela to a disease process in a body compartment other than the foot, it is of paramount importance that the primary disease is treated urgently and effectively. If the duration and severity of the primary disease can be reduced by intensive therapy, there is a strong chance that the severity of lamellar pathology may also be reduced, thus improving the prognosis for the horse. Nevertheless, severe laminitis is sometimes the outcome despite the best of current therapy.

When the laminitis process is triggered, there is virtually nothing, by way of drug therapy, that will stop its relentless progress. The administration of a nonsteroidal anti-inflammatory drug (NSAID) like phenylbutazone, during the developmental/acute stages, will abolish foot pain and create a more comfortable-looking horse, but the disease continues unabated. This creates an ethical dilemma; balancing the need to alleviate pain and suffering against the realisation that most of what is administered is only palliative. When NSAIDs are in use, the patient should be confined to a stall with deep bedding. Exercise, while under the influence of painkillers, such as phenylbutazone, is contraindicated.

Cryotherapy

The results of experiments at the AELRU, continuously evaluating foot temperature (and by implication foot circulation), as horses developed laminitis, showed that vasoconstriction during the developmental stage of laminitis may have had a protective effect (Pollitt and Davies 1998). The induction of digital vasoconstriction may be a useful preventive strategy in the developmental phase of laminitis. Limited anecdotal evidence from practicing veterinarians suggests that cryotherapy may halt the development of the disease. The profound hypometabolic effect of cryotherapy is considered to be the most important mechanism by which cold limits the severity of an injury. Tissue metabolic rate and oxygen consumption are inversely related to temperature. A reduced requirement of cooled tissue for oxygen, glucose and other metabolites enhances the survival of cells during periods of ischemia. This mechanism is thought to protect tissue and is the basis for the use of cryotherapy in organ transplant surgery. A reduction in metabolic enzymatic activity of approximately 50% has been observed with a reduction in tissue temperature of 10°C. The activity of collagenases and pro-inflammatory cytokines is significantly reduced at lower temperatures.

Cryotherapy causes potent local vasoconstriction. This is largely mediated by sympathetic nervous control; however, a direct constrictive effect on blood vessel walls may occur, particularly at lower temperatures. Clinical recommendations for the duration and temperature of cryotherapy in horses are extrapolated from human medicine. Our recent studies have challenged these recommendations.

Table 1 The Potential Mechanisms by Which Continuous Distal Limb Cryotherapy May Prevent Acute Laminitis

Pathophysiological Mechanisms Implicated in the Development of Laminitis	Potential Beneficial Effect of Cryotherapy
Delivery of "laminitis trigger factors" to the digit via the circulation	Profound vasoconstriction limits the delivery of haematogenous "trigger factors" to the digit
Production and activation of excess lamellar matrix metalloproteinases (MMPs)	Profound hypometabolism reduces the production and activation of lamellar MMPs
Enzymatic degradation of lamellar attachments by MMPs	Profound inhibition of enzymatic activity through hypometabolism
Local production and activity of pro-inflammatory cytokines (TNF- α and IL1- β)	Hypometabolic effect reduces the production and activity of cytokines
Inflammatory damage caused by infiltration of polymorphonuclear leukocytes (PMNs)	Vasoconstriction and hypometabolism reduce the delivery and activity of PMNs
Digital hypoperfusion resulting in lamellar ischaemia	Profound hypometabolic effect protects lamellar tissue from ischaemic damage
Reduced cellular glucose availability	Profound hypometabolic effect reduces lamellar requirement for energy substrates

Cryotherapy: Potential Mechanisms for Preventing Lamellar Damage

The precise, molecular pathogenesis of acute laminitis is unknown. The diverse effects of cryotherapy, however, have the potential to interrupt many of the pathophysiological mechanisms that have been hypothesized to occur during the developmental and acute phases of the disease. A summary is presented in Table 1. Enzymatic degradation of lamellar attachments by matrix metalloproteinases (MMPs) forms the basis of our pathophysiological theory for developmental laminitis. It is hypothesized that the inappropriate release of excess, activated MMPs is mediated by "laminitis trigger factors" delivered to the foot via the digital circulation during developmental laminitis. The delivery of these triggers, which may include cytokines, protein fragments or bacterial products of hindgut origin, appear to be limited by cold-induced digital vasoconstriction during the developmental phase of laminitis. This was the basis for evaluating the use of cryotherapy for the prevention of laminitis. The potent local hypometabolic effect of cryotherapy could augment the vasoconstrictive effect on the digital vasculature. A cold-induced reduction in the local production and activity of MMPs would limit degradation of the lamellar attachments. A digital hypometabolic state would also limit the local production and activity of pro-inflammatory cytokines, such as interleukin and tumor necrosis factor, during the developmental stage of laminitis. Cryotherapy could also limit secondary inflammatory damage caused by white blood cell

infiltration. Similar mechanisms are believed to be the basis for the efficacy of scalp cryotherapy in preventing alopecia in cancer patients undergoing chemotherapy. Vasoconstriction apparently reduces delivery of the chemotherapeutic agent to the scalp, and cellular uptake and metabolism are reduced when residual drug reaches the hair follicles.

The alternate pathophysiological theory for laminitis proposes that digital hypoperfusion during the developmental stage leads to lamellar ischemia and necrosis. Profound, cold-induced vasoconstriction would seem contraindicated if digital hypoperfusion was the primary mechanism behind the development of laminitis. However, despite a reduction in digital perfusion, the hypometabolic effect of cryotherapy could protect the lamellar tissue from ischaemic damage. Similarly, a profound cold-induced reduction in metabolism could protect the lamellar tissue from a lack of glucose (proposed as an initiator of lamellar separation in one study). Until the true pathophysiology of laminitis is discovered, the apparent resilience of the equine distal limb to prolonged, extreme cold may hold the key to successfully preventing the disease. Continuous distal limb cryotherapy during the developmental stage of laminitis has the potential to preserve the lamellar tissue until the systemic insult, occurring elsewhere in the body, has abated.

The Efficacy of Continuous Distal Limb Cryotherapy for the Prevention of Acute Laminitis.

Experimental Data. We have completed two controlled studies on the efficacy of cryotherapy for the prevention of laminitis. In the first study laminitis was induced in six horses using the oligofructose overload model (van Eps and Pollitt 2004). Each horse had one forelimb immersed in ice and water (mean temperature 0.5-1.7°C) for a 48 hour experimental period, achieving a mean internal hoof temperature of 3.5-0.9°C. All horses developed clinical and histological laminitis in one or more of the untreated limbs. The cooled limbs did not develop clinical laminitis and had significantly reduced lamellar histological damage. The study also showed significantly reduced up-regulation of lamellar MMP mRNA in the cooled limbs when compared with the untreated limbs.

Although cryotherapy markedly reduced the severity of laminitis it did not completely prevent minor histological changes in 4 of the 6 horses.

In a second study cryotherapy was applied to all 4 limbs of 6 horses for 72 h. Laminitis was induced as before and the observation period was extended until 7 days post oligofructose dosing. The horses showed either no or very mild clinical signs of laminitis and histology of lamellar tissues taken 7 days post induction showed no laminitis. Control horses were lame at 7 days and had moderate to severe laminitis histopathology (van Eps *et al.* 2004).

Cryotherapy was instigated immediately following administration of the carbohydrate induction bolus in these studies. In a clinical case of grain overload or acute colitis such prompt initiation of cryotherapy may not be possible. It is unclear whether such a potent prophylactic effect would occur if cryotherapy was initiated later in the course of the disease when lameness was already present. Thus the potential of cryotherapy to prevent laminitis has been demonstrated and further clinical evaluation of the technique is justified.

Clinical Data

Anecdotal evidence of the successful use of cryotherapy to prevent acute laminitis has surfaced following the initial evidence-based recommendations for its use. The authors have trialed continuous distal limb cryotherapy for the prevention of laminitis in 7 cases of acute colitis (5 Thoroughbred geldings, 1 Thoroughbred colt and 1 Arab mare). All cases presented with fever ($>39.5^{\circ}\text{C}$), profuse watery diarrhea and signs of endotoxaemia and circulatory shock (injected mucous membranes with poor capillary refill time, rapid heart rate and depression). Only one horse had signs of laminitis before the initiation of cryotherapy. This horse had increased intensity of digital pulses in all four limbs, though lameness was not obvious. All cases were placed into a plastic tub with a rubber floor. Shoes, if present, were not removed. Water, then cubed ice, was added to the tub to submerge the fore and hind limbs. The level of ice and water was maintained at the upper third of the cannon bones. Approximately 100 kg of cubed ice

was required to cool the water initially. Subsequently, 50 kg of ice was added at 4- to 8-hour intervals to maintain the temperature within the bath at less than 5°C.

All horses were treated (while in the cold bath) with intravenous polyionic fluids and plasma, antibiotics, NSAIDs and activated charcoal and paraffin oil by nasogastric tube. Lucerne hay and water were provided *ad libitum*. The cases were monitored constantly and remained in the cold bath for a minimum of 72 hours. All horses tolerated the cold bath well, without attempting to escape. The decision to remove the horses from the cold bath after the 72-hour period was based on resolution of clinical signs. Each horse was removed when the rectal temperature stabilized below 38.5°C, the manure was formed, and the mucous membranes returned to normal color. Five of the horses were removed at, or shortly after, 72 hours. The remaining 2 horses were removed from the bath at approximately 96 hours. None of the horses were lame on removal from the cold bath; however, all had increased intensity of digital pulses in all four limbs for the ensuing 24 hours. Variable distal limb oedema was also present. One horse that had signs of incipient laminitis before commencement of cryotherapy was mildly lame between 12 and 24 hours after removal from the cold bath. The lameness disappeared over the subsequent 10 days of hospitalization and radiographs of this horse revealed no displacement of the distal phalanx within the hoof capsule. It is unclear whether cryotherapy reduced the severity or had no effect on the development of laminitis in this case.

The remaining 6 horses were sound throughout the hospitalization period, and no lameness was detected on subsequent re-examinations 4 to 6 weeks later. All horses have returned to athletic activity, reportedly at previous levels. At the time of publication, three of the Thoroughbred horses have won metropolitan races since discharge. After examination of hospital records, the authors estimate the incidence of acute laminitis in previous similar cases of acute colitis (that were not treated with cryotherapy) to be 40 to 50%. Although these are very limited numbers, the authors believe the prophylactic use of continuous distal limb cryotherapy in similar cases at risk of developing laminitis is worthy of further clinical evaluation.

Current Recommendations

Case Selection

Any horse that is at immediate risk of developing acute laminitis is an appropriate candidate for continuous distal limb cryotherapy. Deciding which cases are at risk of developing acute laminitis is largely a matter of clinical experience. Primary diseases known to predispose horses to acute laminitis include colitis, proximal enteritis, metritis, pleuropneumonia, myositis, peritonitis, and complicated colic.

Timing and Duration

The target period for distal limb cryotherapy is the developmental phase of laminitis. This has been defined as the period between the initial causative insult and the first appearance of acute lameness identifiable as laminitis. The duration of the developmental phase is highly variable. In experimentally induced cases of carbohydrate overload, the developmental phase appears to last between 20 and 72 hours, with an average duration of approximately 40 hours. A similar duration appears to exist in clinical cases of acute bacterial colitis. A longer and more variable duration may exist in association with other diseases such as pleuropneumonia. It is currently impossible to accurately predict the development of laminitis in individual cases, and therefore it is impossible to determine that a horse is within the developmental phase of laminitis. Initiation of cryotherapy must therefore be based on a high probability that a horse is within the developmental phase of laminitis; ie, the horse has no clinical signs of laminitis but is considered at high risk of imminent development of the disease. There is currently no evidence to support the initiation of cryotherapy in horses with pre-existing clinical signs of laminitis (horses that have progressed to the acute phase of laminitis).

Ideally cryotherapy should be applied for the entire duration of the developmental phase of laminitis. The maximum duration of the developmental phase in experimentally induced cases of carbohydrate overload appears to be 72 hours; ie, horses that do not have acute laminitis by 72 hours after administration of carbohydrate do not develop the disease. This can be used as a guide for the duration of cryotherapy application in clinical cases of carbohydrate overload. Resolution of the primary disease may be used

as an indicator for timing the cessation of cryotherapy in individual cases. Although no direct link between endotoxin and laminitis has been established, clinical signs of endotoxaemia usually accompany clinical and experimentally-induced cases of acute laminitis. Abatement of clinical and laboratory signs of endotoxaemia can be used as a marker for cessation of distal limb cryotherapy in many cases. We suggest the continuation of cryotherapy for approximately 12 hours after the signs of endotoxaemia have abated. The authors have maintained continuous distal-limb cryotherapy for a maximum of 7 days without apparent complications. We do not suggest the intermittent use of cryotherapy to prevent laminitis. Vasodilation appears to follow intermittent cryotherapy, and could potentially be detrimental. The authors have noted profound reflex vasodilation in experimental and clinical cases after cessation of cryotherapy. This is manifest as an increase in the amplitude of digital pulses and warming of the feet. The phenomenon lasts between 12 and 24 hours after removal of cryotherapy and could be confused with the onset of acute laminitis. Horses should be closely monitored during this period for the development of lameness typical of acute laminitis. Acute laminitis should be appropriately treated using conventional therapy.

Application Methods

Any means by which the distal limbs can be continually exposed to temperatures of 0 to 5°C is acceptable. The cooling method should include the hoof and its solar surface. We suggest cooling the limb up to the top of the cannon, as this appears to result in more effective cooling of the lamellar region. Cooling just the feet is not enough. Ice and water immersion is effective, practical and inexpensive. Commercial cryotherapy cuff devices could be modified to include the hoof, though this is practically difficult. These devices are usually designed for compression as well as cooling. The effects of prolonged compression on the equine distal limb are currently unknown. The authors have had experience with a range of boots and tubs for ice and water immersion (Fig 1).



Figure 1. Methods of distal limb cryotherapy application. Freestanding (A) and fixed (B) boots for ice and water immersion. These devices are extremely difficult to apply to the hind limbs. A tub, filled with ice and water to a level just distal to the carpus, is the most practical means of cryotherapy application to all four limbs in the authors' experience (C). This clinical case of acute colitis remained in the cold bath constantly for 72 hours and did not develop acute laminitis. With permission: A.W. van Eps et al (2004) Distal Limb Cryotherapy for the Prevention of Acute Laminitis. Clin Tech Equine Prac 3:64-70.

We have found that the use of a tub, 200 cm long, 80 cm wide and 50 cm high, most practical for prolonged, continuous application of cryotherapy to all four limbs. A water-tight door at one end for ease of access, and a rubber floor are suggested (Fig 2). Temporary or permanent stocks, together with cross-tying the head may assist in keeping the horse stationary. A refrigerated pump, re-circulating water at around 2°C, can reduce or replace the requirement for ice. Overall, vigilance should be exercised to maintain immersion temperatures below 5°C to maximize the protective effect.



Figure 2. Experimental set-up showing a horse is restrained standing in a tub of marine ply construction for prolonged, continuous application of cryotherapy to all four limbs. There is a water-tight door at one end for ease of access and a rubber floor. The horse is cross-tied standing in cold water to the proximal cannon level. A refrigeration unit and pump, against the far wall, are cooling and re-circulating water at around 2°C. Plastic sheeting prevents food, faeces and urine from contaminating the water and blocking the pump filters.

Conclusion

Continuous distal limb cryotherapy shows considerable promise as a technique for preventing acute laminitis. The authors continue to evaluate cryotherapy in clinical cases

at risk of developing laminitis, and welcome correspondence from others engaged in similar pursuits. Currently the most challenging aspect of cryotherapy in the clinical situation is the identification of cases that will develop laminitis, and subsequently deciding when to initiate and cease cryotherapy in these cases. A biological marker to identify horses at imminent risk of developing laminitis is needed. Such a marker would define the clinically silent developmental phase of laminitis in individual cases, and greatly improve the potential for prevention of the acute disease. Undoubtedly genetic markers exist for the early identification of horses developing laminitis. Up-regulation of MMP-2 mRNA early in the acute phase of laminitis has been demonstrated in lamellar tissue. If this process begins during the developmental phase of laminitis, particularly within the blood, skin, ergot or chestnut tissue, a diagnostic potential exists. The eventual discovery of the exact pathophysiology of laminitis will surely lead to effective and direct methods of prevention and therapy. In the meantime, the apparent resilience of the equine distal limb to prolonged, extreme cold can be harnessed and may hold the key to successfully preventing the disease.

(Modified from the PhD studies of Andrew van Eps and the publication “Distal Limb Cryotherapy for the Prevention of Acute Laminitis” (2004) by Andrew W. van Eps, Lester J. Walters*, Gregory I. Baldwin*, Mark McGarry* and Christopher C. Pollitt. In: Clinical Techniques in Equine Practice 3:64-70. * Eagle Farm Equine Veterinary Hospital, Hamilton, Queensland.

Management practices to avoid pasture associated laminitis.

There is now strong circumstantial evidence that fructan in the hindgut of horses may trigger laminitis. Horses can ingest fructan rich pasture rapidly in amounts exceeding (Longland and Byrde 2006) that used to induce experimental laminitis (van Eps and Pollitt 2006).

Owners of horses predisposed to laminitis should develop strategies to reduce risk. Most horse owners in New Zealand and Australia are committed to pasture feeding regimens throughout the year so a combination of both pasture and horse management practices need to be considered. The aim is to reduce the concentrations of water soluble carbohydrate (WSC) in pasture and to prevent its consumption by the grazing horse.

Pasture factors

Some pasture species are notorious fructan accumulators (they are selected and bred for this) and if possible should not be fed to horses. The WSC content of grass can reach 56% of its total dry matter (DM) of which fructan can be 44%. Grass that is actively growing tends to store less WSC. Maintaining soil moisture and fertility and keeping grass short by mowing or grazing encourages leaf growth and WSC consumption (Watts and Chatterton 2004). WSC accumulation in grass is driven by photosynthesis and takes time to occur. It peaks in the afternoon and early evening and high WSC intake can be avoided by allowing grazing only in the early morning. Likewise pasture shaded by tree-lines and windbreaks accumulates less WSC and susceptible horses can be strip grazed behind electric fences in these areas. Some horse managers will poison selected paddocks to eliminate pasture altogether and at time of high risk keep their horses on these “dry lots”. Times of risk are conditions of high light intensity and low ground temperatures such as in spring and autumn and particular care is indicated at these times. Under these conditions photosynthesis and WSC production is relentless but growth and metabolism is slow; hence WSC accumulation. Using a cash flow analogy; the bank balance is greatly in credit – cash income exceeds expenditure. Drought or periods of low soil moisture may also drive WSC accumulation and even dry looking pasture can have a high WSC concentration. Drought breaking rain can also be a trap. WSC accumulated in subsoil roots during dry times is rapidly mobilized to new shoots and many a pony has foundered on insignificant looking pasture after rain. Another trap is slashed or heavily grazed pasture or stubble after harvesting. Most of the WSC of grass is stored, not in the green leaves, but in the lower, pale green stems that as a WSC reservoir. Grass that has gone to seed in summer is usually low in overall WSC content in its leafy tissues but could still pose a risk from the starch in the seeds. A yield of starch from the seed of perennial ryegrass has been estimated at 360kg/ha per growing season (Longland and Byrde 2006). Horses will selectively strip seed from standing pasture and could conceivably consume sufficient starch to trigger laminitis from hindgut fermentation.

Horse factors

Grazing muzzles have been successfully used to limit grass and thus WSC intake by horses at pasture. The hole in the muzzle limits intake and confines it to leafy tops that are lower in WSC content (Fig 3).



Fig 3. Pony fitted with a grazing muzzle. Grazing through the hole in the base of the muzzle (inset) limits the amount of pasture consumed to safer levels.

When horses and ponies have no access to pasture and are yarded or confined to dry lots what are they to be fed? The usual solution is grass or forage hay. However the haymaking process may not always reduce WSC and sometimes the most innocent looking hay may have dangerous WSC levels. If possible choose hay made from mature seeded, pasture made in summer. Hay could still be dangerous if harvested during periods of plant stress such as autumn and spring. Analysis of the WSC content of such hay is warranted but not always practical. Fortunately soaking hay in fresh water leaches out WSC (but not starch) and reduces the WSC content significantly. Sixty minutes of soaking and draining removed an average of 31% of the soluble sugars from 15 hay samples (Watts and Chatterton 2004).

Pony breeds in particular are prone to obesity and insulin resistance and obese individuals are at high risk of developing laminitis. The diet of obese individuals can be modified so that energy is derived from fat and fibre rather than from high glycaemic sources.

Owners should monitor the body weight and learn to condition score their horses aiming for more optimum weights. Insulin resistance can be reversed by weight reduction and regular exercise.

“Founderguard” is an antibiotic formulation that can be fed to horses and ponies at pasture and when present in the hindgut limits the proliferation of *Streptococcus bovis*. When ‘predosed’ it may control hindgut carbohydrate fermentation to levels that prevent serious laminitis.

Conclusion

Half of laminitis science is incorrect – but which half? Out of the nebulous laminitis data of the last 60 years patterns and trends are appearing from which principles are crystallizing into veterinary consciousness. Although we have made some progress towards achieving the goal of the AELRU there are still gaps in our knowledge. The biological basis of laminitis has become molecular and the discipline of molecular biology has laminitis in its cross-hairs. These are exciting times to be involved in equine research – we now have tools our forefathers would not have thought possible. A coherent body of knowledge will soon emerge that will demystify laminitis.

Ethics statement

All experiments on horses, conducted by AELRU, are approved by The University of Queensland Animal Ethics Committee (constituted as per the National

Health and Medical Research Councils "*Australian code of practice for the care and use of animals for scientific purposes*" which is embedded in the "*Queensland animal care and protection Act 2001*") and all horses under experimentation are inspected by an Animal Welfare Officer.

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