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Update on the pathophysiology of laminitis

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INTRODUCTION

Much has been written on the subject of equine laminitis and the disease does not appear to fit into any familiar, disease-causing pattern, despite, years of attempts to fit this very square peg into several round holes. What is required is an understanding of the disease process so that preventive, even curative, strategies can be developed. We need to ask “why” rather than “how” laminitis develops. Laminitis appears to be less of a disease, but more of a natural process gone wrong. Rather like the workings of the worst malignant cancer; a process that still defeats the best human medical minds.

GRASS FOUNDER

Ponies, and occasionally horses, will develop laminitis or “grass founder” while grazing on pasture. Under certain conditions of climate, a soluble sugar called fructan may reach very high concentrations in the stem of grass (up to 50% dry matter). When consumed, fructan (or oligofructose) is rapidly fermented by hindgut microorganisms, triggering a gastrointestinal disturbance that somehow leads to laminitis. Mammals have no enzyme to digest fructan, so when consumed it passes undigested into the caecum where it undergoes rapid microbial fermentation causing a population explosion of hindgut streptococci.

GRAIN FOUNDER

In field cases, grain founder occurs following the consumption of excessive amounts of grain, either from accidental access by the horse or by a misguided, intentional, dietary increase by its keeper. Although the amount of grain required to induce laminitis varies between individuals, the consumption of 5-8 kg of wheat grain by the average 400-450kg horse causes faecal acidity (pH 4-5 instead of the normal 6.8-7.5), lactic acidemia, profuse watery diarrhoea and fever, all of which are associated with laminitis.

ALIMENTARY CARBOHYDRATE OVERLOAD MODEL

Much of what we know about laminitis and the metabolic events surrounding it has been derived from studies on horses that have been experimentally dosed with excess soluble carbohydrate (the alimentary carbohydrate overload model). Upon mixing with the normally neutral caecal contents, excess starch or fructan undergoes rapid fermentation to lactic acid. With the arrival of more and more substrate, fermentation continues and the unnaturally acidic conditions favour the rapid proliferation of equine hindgut streptococcal species or EHSS (Milinovich et al. 2008; Milinovich et al. 2007; Milinovich et al. 2006). The predominant organism is Streptococcus lutetensis (formerly S.bovis). EHSS proliferation results in very acidic conditions in the hindgut with pH as low as 4. Two isomers of lactic acid, D and L-lactate, are produced in almost equal proportions by EHSS fermentation in the equine hindgut. However, only L-lactate is produced by the metabolic activities of mammals, so the concentration of D-lactate in venous blood can be used as an accurate indicator of EHSS fermentative activity in the hindgut (van Eps and Pollitt 2006). Low pH in the large intestine initiates a series of secondary events that often, but not always, culminate in laminitis. One of the most important consequences is the death en masse and lysis of large numbers of EHSS and the release of the toxic components of their cell walls and genetic material (endotoxins, exotoxins, microbial DNA and phage). Toxins absorbed from the gut into the bloodstream during developmental laminitis and toxaemia following alimentary carbohydrate overload creates a very severe illness for the horse. Interestingly, experimental administration of endotoxin itself has never been able to cause laminitis. In addition, endotoxaemia can be effectively controlled by a range of drugs (e.g. polymyxin B, flunixin meglumine [Finadyne]) and laminitis develops regardless of their use.

As early as 24 hours after carbohydrate overload, the epithelial cells lining the caecum undergo degenerative changes and the bowel becomes leaky. By 48-72 hours there is widespread desquamation and sloughing of caecal epithelial cells sufficient to allow passage of lactic acid, toxins and laminitis trigger factors into the circulation. The consequences can be catastrophic. About 10-15% of horses die from cardiovascular shock after the accidental consumption of excess grain. High heart rates, rapid breathing, fever, sweating, colic, diarrhoea and depression are the signs of horses battling grain overload. Just when the horse turns the corner and responds to treatment and the severity of the clinical signs decreases, the signs of foot pain appear; laminitis has arrived on the scene.
VASODILATION IS ASSOCIATED WITH LAMINITIS

To determine if it was lamellar vasoconstriction or vasodilation which preceded laminitis, lamellar hoof temperature was measured continuously in horses developing laminitis (Pollitt and Davies 1998). Variations in hoof temperature were assumed to signify fluctuations in lamellar blood flow. The unambiguous presence or absence of laminitis was based on histopathological grading of lamellar tissues after euthanasia. Analysis of mean hoof temperature graphs showed that the 6 horses judged laminitis positive had experienced a period of prolonged digital vasodilation during the developmental phase. The 8 laminitis negative horses experienced no such period of vasodilation and had hoof temperatures never significantly above that of normal horses. Despite the horses appearing equally ill with similar clinical signs of fever, gut stasis (paralytic ileus), diarrhoea, elevated heart rate and low faecal pH, the only parameter which significantly differentiated the laminitis positive from laminitis negative horses, during the developmental phase was foot temperature. Thus for laminitis to occur, a period of sublamellar vasodilation during the developmental phase had occurred. If the digital circulation was vasoconstricted during this period then laminitis did not occur.

It was assumed that the period of increased digital perfusion in laminitis positive horses, concomitant with the severe metabolic crisis brought on by alimentary carbohydrate overload, metritis/retained placenta or pleuropneumonia exposed lamellar tissues to a concentration of blood borne factors sufficient to trigger lamellar separation. A hypothesis that lamellar separation could occur if uncontrolled MMP activation damaged the lamellar basement membrane was developed. Evidence that metalloproteinase production increased and metalloproteinase activation occurred was required for this theory to be validated.

THE LAMINITIS LESION

In acute laminitis, the tissue suspending the distal phalanx from the inner hoof wall fails, specifically at the junction between the connective tissue of the dermis or corium (the bone side) and the basal cell layer of the epidermal lamellae (the hoof side). This junction, the basement membrane zone, appears to be the weak link in an otherwise robust and reliable structure. In acute laminitis there is wholesale epidermal cell detachment from, and lysis of, the lamellar basement membrane and this leads to failure of the lamellar anatomy and, ultimately, failure of the distal phalanx suspensory apparatus. There is a good correlation between the grade of severity, as seen with the microscope (histopathology), and the degree of lameness (using the Obel grading system) shown by the horse (Pollitt 1996). Thus, when the horse first starts to show the foot pain of laminitis, it means that the anatomy of the hoof wall lamellae is being destroyed: the worse the lameness, the worse the damage. Any activity that places stress on an already weakened lamellar attachment apparatus (such as forced exercise and distal limb nerve blockade) will cause further damage and is contraindicated.

THE LAMINITIS PROCESS

The spectacular disintegration of the lamellar attachment apparatus, initiated during the developmental phase of laminitis, renders a normally robust and trouble-free epidermal/dermal system useless in a relatively short period of time. Logic indicates that somehow this normally tightly-controlled, metabolic process is thrown into disarray to cause the lamellar specific lesion during the laminitis developmental period. Our evidence suggests that it is the enzymatic remodelling of the epidermal lamellae, assumed to be essential if the continually proliferating hoof wall is to move past the stationary distal phalanx, that is activated beyond control to destroy the lamellar attachment apparatus. The enzymes that destroy the key components of the lamellar attachment apparatus are metalloproteinase-2 and metalloproteinase-14 (MMP-2 and MMP-14). These enzymes are also found in a wide range of other remodelling tissues such as bone, joints and endometrium, as well as in metastasizing malignant tumours. An additional metalloproteinase, MMP-9, is also present in laminitis affected tissue but this seems to be derived from white blood cells that have been attracted to the lamellar zone during laminitis development (Black et al. 2006). Normal MMP activity is constantly responding to the stresses and strains of equine life as well as to constant growth. When called for, sufficient MMP is manufactured locally to release epidermal cell to cell, and cell to basement membrane attachment, as required, maintaining the correct shape and orientation of the lamellae. From time to time, injury to the basement membrane would require its lysis and reconstruction. The controlled release of tissue inhibitors of MMP (TIMPs) keeps the remodelling process in equilibrium. The hoof lamellae and the hoof itself slowly migrate past the stationary basement membrane that is firmly attached to the connective tissue covering the upper surface of the distal phalanx.

The sequences of microscopic events that initiate laminitis follow a consistent pattern and the stages of histological laminitis were graded according to the degree of severity of these changes (Pollitt 1996). The lamellar basement membrane (BM) was the key structure affected and since the BM bridges the epidermis of the
hoof to the connective tissue of the distal phalanx, it follows that the wholesale loss and disorganization of the lamellar BM inexorably leads to the failure of hoof anatomy so characteristic of equine laminitis. Loss of hemidesmosomes (HDs), cleavage of laminin-5 anchoring filaments and BM nanoblisters are present 24h post dosing (French and Pollitt 2004; Nourian et al. 2007). Uncontrolled activation of lamellar MMP is the likely cause of the anchoring filament destruction. When the numbers of HDs in the BM zone are counted there is a significant correlation between HD density and clinical severity of laminitis.

**LAMINITIS CAUSES INCREASED GENE TRANSCRIPTION OF LAMELLAR ENZYMES**

Destruction and detachment of the lamellar basement membrane are the key lesions of acute laminitis. The genes controlling hoof lamellar MMP activity are significantly upregulated in tissues affected by acute laminitis, thus providing firm circumstantial evidence that MMP activation is a pivotal event in the development of laminitis (Kyaw-Tanner et al. 2008; Kyaw Tanner and Pollitt 2004). ADAMTS-4, an additional enzyme, capable of attacking other components of the lamellar BM zone, is also significantly up-regulated by laminitis (Coyne et al. 2008).

**METALLOPROTEINASE INHIBITORS**

The activity of tissue MMPs correlates strongly with the degree of malignancy and invasiveness of lethal human tumours, such as malignant melanoma, breast and colon cancer. Degradation of the proteoglycan present within articular cartilage caused by ADAMTS-4 is a feature of osteoarthritis. Research in these fields has generated a wide range of chemical agents capable of inhibiting enzyme activity both in vitro and in vivo. We have shown that one of these (Batimastat or BB-94, British Biotech, Oxford) blocks the activity of the laminitis MMPs in vitro and has the potential to be a useful tool in the prevention and management of acute laminitis (Pollitt et al. 1998). We are conducting trials to test whether MMP inhibitors can prevent or ameliorate field cases of laminitis.

**EQUINE METABOLIC SYNDROME**

The term equine metabolic syndrome refers to horses with a history of laminitis, insulin resistance, cresty necks and increased adipose tissue deposits in the withers and dorsal area of the back (Johnson 2002). Elevated serum insulin concentrations distinguish ponies that are susceptible to dietary pasture associated laminitis (Treiber et al. 2006a; Treiber et al. 2006c). Furthermore, insulin concentrations are markedly elevated in ponies that develop laminitis after grazing high carbohydrate pasture, while glucose, free fatty acid and cortisol concentrations remain normal (Treiber et al. 2006b). In contrast to humans, insulin-resistant horses rarely develop pancreatic exhaustion and are capable of producing exceptionally high serum insulin concentrations (McGowan et al. 2004; Reeves et al. 2001). Insulin toxicity appeared to be a key factor in triggering equine laminitis. The onset of laminitis is associated with plasma insulin that exceeds 100 µIU/ml [normal range = 8 to 30 µIU/ml] (Walsh et al. 2007).

**INSULIN ALONE CAUSES LAMINITIS**

To test the hypothesis that hyperinsulinaemia triggers laminitis, normal, lean ponies, with no prior history of insulin resistance or laminitis, were subjected to prolonged hyperinsulinaemia and euglycaemia. All of the ponies developed laminitis within 72h of hyperinsulinaemia (Asplin et al. 2007). This highlights the importance of insulin in the pathogenesis of endocrinopathic laminitis. Interestingly the histopathology and ultrastructure of insulin induced laminitis was markedly different from carbohydrate induced laminitis; there was scant BM separation but excessive lengthening and attenuation of secondary epidermal lamellae. Insulin appeared to have exerted a profound mitogenic affect on the lamellae weakening them to the point of failure. Early control of circulating insulin may halt or even reverse this process. Ponies at risk of laminitis should be blood tested for the detection of hyperinsulinaemia. Prior to sampling access to grain or other soluble carbohydrate must be prevented for 3 hours. A single blood sample showing elevated insulin predicts that laminitis will occur or may become worse (Walsh et al. 2007). Techniques should be employed to lower insulin concentrations and restore insulin sensitivity. A weight reducing diet with a low glycaemic index and physical exercise reduce insulin resistance in horses (Pratt et al. 2006). Further work is required to see if insulin-sensitising drugs of the type given to human patients with Type 2 diabetes will be effective in insulin resistant horses.

**SUPPORTING LIMB LAMINITIS**

Laminitis in the lamellae of a single hoof can occur whenever a horse’s limb is forced to bear weight uni-laterally for prolonged periods of time. This can occur when an injury (bone or joint fracture) or disease process (septic arthritis) in the contralateral limb is so painful that weight bearing is impossible. After 2-3
days of unrelieved weight bearing, the supporting limb develops lamellar pathology, often to a severe degree. The case for ischaemia as the cause of supporting limb laminitis appears to be clear cut. The evidence comes from in vitro studies using digitised subtraction angiography (DSA) in isolated perfused horse limbs (obtained after humane slaughter at a knackery). When a mechanical press was used, to place the limb in the loaded, (fetlock fully extended) position, there was zero perfusion of the foot below the level of the coronary band. When the limb was not loaded perfusion through all the major vessels of the foot was normal. Presumably a similar situation prevails in vivo and chronic lack of perfusion eventually triggers a lamellar pathology indistinguishable from that initiated by other causes. This form of laminitis may be prevented if the supporting limb is firmly wrapped in an elastic support bandage and shod with an effective support shoe. The horse should be provided with a deep bed of wood shavings or sand so that it can lie down comfortably and allow blood to circulate through its feet. Deep, compliant bedding also allows the horse to find a foot position that promotes the circulation. The injured limb should be treated promptly and fitted with a cast or splint so that it can begin to take its share of weight bearing. Pain should be controlled with analgesics for the same reason.

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