

A review of recent developments in the pharmacological prevention and treatment of endocrinopathic laminitis

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Abstract. Despite the prevalence of endocrinopathic laminitis, the pharmacologic options for preventing and treating the disease are severely limited. The present review aims to discuss the spectrum of potential therapeutic agents for the condition, ranging from early experimental compounds to agents nearing registration. There are different pharmacologic targets for, and approaches to, managing laminitis. Reducing hyperinsulinaemia is central to diminishing endocrinopathic laminitis risk, and a detailed understanding of the pathophysiology of insulin dysregulation is necessary to identify pathways that can be targeted to minimise post-prandial insulin secretion and action. This area of research is advancing rapidly, with several exciting prospects, such as sodium-dependent glucose co-transporter-2 inhibitors, on the horizon for the treatment of equine metabolic dysfunction. Drugs that directly target the lamellae and aim to reduce the damage inflicted on the lamellae as part of this condition, are not yet available. Although progress in this area of laminitis therapy is slower, improved understanding of the events that lead to lamellar failure has enabled the investigation of novel drugs that aim to prevent laminitis at the site of the lesion. Finally, a brief review is included of the directions being taken in the management of the chronic and acute pain that accompanies laminitis. Medications for relieving the pain associated with laminitis are currently the most-prescribed drugs for the disease, and range from simple, affordable and thoroughly tested options, such as phenylbutazone, to newer, less-understood applications such as paracetamol and gabapentin. In the future, endocrinopathic laminitis management plans will likely take a multi-faceted approach that still hinge on effective dietary management and exercise, but also include drugs that address foot pathology, pain and underlying endocrine disturbances.

Additional keywords: equine metabolic syndrome, horse, hyperinsulinaemia, insulin dysregulation, lamellae, pituitary *pars intermedia* dysfunction.

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Introduction

Laminitis persists as one of the greatest challenges in veterinary medicine, not only for the owner faced with managing their animal through laminitis episodes and endeavouring to reduce the risk of recurrence, but also for the clinician seeking to manage the condition, and for the researcher seeking to understand the complex pathophysiology of the disease. There are three principal forms of laminitis, namely, sepsis-related, supporting limb and endocrinopathic (Belknap 2016). Sepsis-related and supporting limb laminitis are likely to involve differing aetiological pathways to endocrinopathic laminitis, and, therefore, alternative medications may be appropriate for treating these forms of the disease. This review focusses solely on recent developments in the pharmacological prevention and treatment of endocrinopathic laminitis, the most common variant of the disease (Karikoski *et al.* 2011).

A fundamental step towards preventing or treating endocrinopathic laminitis is to understand its pathophysiology. After more than a decade of searching for causality among

numerous effects, including altered blood flow, gastrointestinal disturbances, the release of bacterial toxins, the generation of vasoactive amines and matrix metalloproteinase (MMP) enzyme activation, the demonstration that laminitis can be induced in healthy ponies and horses through the prolonged infusion of insulin and glucose (Asplin *et al.* 2007; de Laat *et al.* 2010) proved to be an important turning point. Since then, in an effort to improve therapeutic success, several studies have focussed on the management of hyperinsulinaemia, although not every approach is likely to be equally effective.

Both human and equine studies on hyperinsulinaemia associated with metabolic syndrome have classically regarded the condition as a symptom of insulin resistance. In this scenario, the excessive consumption of non-structural carbohydrates leads to a prolonged influx of glucose into the bloodstream. This places constant pressure on the insulin-sensitive glucose transporters (GLUT4) that are responsible for glucose uptake into peripheral tissues (Joost and Thorens 2001). Over time, the GLUT4 transporters become exhausted

and resistant to activation by insulin, such that higher insulin concentrations are required to drive them and maintain euglycaemia (Bloomgarden 1998). In turn, the need for excess insulin production eventually leads to pancreatic failure and Type-2 diabetes mellitus (T2DM: in humans), or else, the insulin reaches toxic levels in the blood and causes laminitis (in horses). However, in 2016 we examined 22 insulin-dysregulated ponies, and found that only 15 were insulin-resistant (de Laat *et al.* 2016). This observation led us to propose that equine insulin resistance may develop after a prolonged period of hyperinsulinaemia and could, in some instances, be a symptom of the condition, rather than a cause. Thus, instead of commencing with a failure of GLUT4 transporters, the development of insulin resistance could be a counter-regulatory response to prolonged hyperinsulinaemia.

As a further advance, we have proposed that the root cause of hyperinsulinaemia is an increased capacity to absorb dietary glucose from the gut, coupled with, under certain circumstances, the actions of incretin hormones such as GLP-1 (de Laat *et al.* 2016) and GLP-2 (de Laat *et al.* 2018a). This effect may not be immediately obvious, as the resulting increase in blood glucose concentrations are subtle. However, this change can be amplified by an increase in insulin secretion in the order of 83%, which is more readily detectable by clinicians (Meier *et al.* 2018b). This new paradigm in the study of insulin dysregulation (ID) has encouraged the move by researchers and clinicians away from commencing an investigation of metabolic dysfunction with intravenous tests of tissue insulin sensitivity. Instead, oral glucose (sugar) tests that include an assessment of the enteroinsular axis are now favoured (Durham *et al.* 2019). Further, if one of the underlying causes of hyperinsulinaemia is the excessive absorption of glucose into the blood stream, then a logical approach to treating ID and preventing laminitis would be to either reduce glucose absorption, or increase the rate of glucose disposal. This knowledge has stimulated increased research on potential therapeutic targets for reducing glucose uptake, hyperinsulinaemia and, ultimately, laminitis. In addition, non-pharmacologic strategies to improve insulin sensitivity, such as reduced caloric intake and exercise (Morgan *et al.* 2016), and pharmacologic agents, such as insulin-sensitising drugs, have received considerable attention (Durham *et al.* 2008).

Treating endocrine disease

As hyperinsulinaemia is the major stimulus for the onset of lamellar pathology and failure, reducing insulin concentrations is crucial for the successful management of endocrinopathic laminitis. As yet, there are no registered pharmacologic agents available for the treatment of ID. Currently, the mainstays of treatment are the non-pharmacologic approaches of dietary restriction and exercise, and their importance should not be under-estimated. Reducing non-structural carbohydrate intake where it is inappropriately high and promoting exercise (where appropriate) to improve tissue sensitivity to insulin are indispensable when approaching the management of metabolic dysfunction. However, as a complementary approach to the management of ID and the prevention of laminitis, substantial research is being invested in the development of new drugs for

treating metabolic dysfunction in horses, with the ultimate aim to reduce laminitis incidence. These pharmacologic options will not be a simple panacea for treating metabolic disease but may prove to be useful, especially for cases that are refractory to management changes or where their instigation is not possible.

Given that the indiscriminate inhibition of insulin receptors is neither practical nor desirable, the focus of drug research on ID has been to reduce insulin secretion. Glucose and the incretin hormones (chiefly GLP-1) are the main catalysts for insulin secretion as described above (de Laat *et al.* 2016). Some ponies with ID appear to have an enhanced capacity for glucose uptake, in addition to upregulated incretin action. Thus, preventing excessive insulin release in response to oral carbohydrates in these animals can, theoretically, be achieved by reducing glucose absorption and hyperglycaemia, and by reducing incretin action. While diminishing glycaemic responses is also a treatment goal in other species, such as in human patients with T2DM, stimulating incretin action, as is practiced in human medicine, would be counter-productive. As a result, oral antihyperglycaemic agents such as metformin and the 'gliflozins' could be usefully adapted for the treatment of horses. Conversely, drugs that mimic or preserve incretin action, such as exenatide and the 'gliptins', would appear to be of little use for the equine species as they exacerbate hyperinsulinaemia, and experimental compounds with opposing actions need to be investigated.

Metformin

The oral biguanide drug metformin is probably the most commonly prescribed medication for ID, although its use in a veterinary context remains off-label. With a multi-faceted mechanism of action, metformin achieves glycaemic modulation by targeting both the liver and intestine. Reduced hepatic gluconeogenesis is coupled with increased small intestinal glucose absorption and utilisation, to achieve an antihyperglycaemic effect in human patients with T2DM. However, in horses, it is likely that any reduction in hyperglycaemia is mediated primarily through the intestinal pathway, as oral bioavailability is low (Hustace *et al.* 2009). Metformin appears to be sequestered in enterocytes where it upregulates anaerobic glucose metabolism and promotes an overall glucose-lowering effect (Wilcock and Bailey 1994). Other potential pathways for the glucose-lowering effect in the intestine have also been proposed, but this important component of the drug's action is still largely unclear (McCreight *et al.* 2016).

Studies of metformin efficacy in horses have produced inconsistent outcomes (Durham *et al.* 2008; Tinworth *et al.* 2012; Rendle *et al.* 2013). These studies employed different methods for assessing insulin regulation, with little response to metformin apparent when intravenous tests of tissue insulin sensitivity were used, and more positive effects on the improvement of ID when oral tests included the enteroinsular axis. These outcomes provide further support that metformin's mechanism of action in horses is primarily intestinal. From a pharmacokinetic perspective, the drug is hydrophilic and its uptake is saturable, suggesting a transporter-dependent

mechanism of absorption. This fits with recent data showing that deletions in certain genes that encode drug transporters impede drug uptake in humans (Markowicz-Piasecka *et al.* 2017). The variable efficacy in equine studies (and clinical outcomes) could, therefore, be due partly to impaired drug uptake by enterocytes in some individuals.

The initiation of metformin treatment usually precedes insulin replacement or supplementation in patients with T2DM, and combined therapeutic approaches that incorporate multiple antihyperglycaemic drugs are increasing in popularity (Cahn and Cefalu 2016). Commonly prescribed second- and third-line agents include incretin-based therapies, sodium–glucose-linked cotransport-2 (SGLT2) inhibitors (see below) and sulfonylureas. While incretin-based therapies in human medicine are not directly translatable to equine ID, the combination of metformin with other compounds that aim to reduce glucose (and ultimately insulin) concentrations, such as a combination of metformin and an SGLT2 inhibitor, is a feasible but unexplored area of veterinary medicine. Recently, a study examined the use of a combination of metformin and sitagliptin (which are formulated together for the treatment of T2DM in people) in six horses (Cárceles-Rodríguez *et al.* 2019). The study concluded that this combination may be beneficial for the treatment of ID, but sitagliptin inhibits the breakdown of aGLP-1 and, therefore, increases incretin action and insulin release, which would presumably increase the risk of laminitis.

SGLT2 inhibitors

When peripheral GLUT4 transporters are working to capacity, little or no glucose is eliminated via other routes such as the kidneys (Poirée *et al.* 1978). However, if excess glucose absorption cannot be avoided, then a different strategy to deal with the problem is to promote glucose disposal via the kidneys. Indeed, such a strategy recently became the foundation for a newer class of therapeutic drugs that have gained popularity for treating human T2DM. The SGLT2 inhibitors were developed from the compound phlorizin, a naturally occurring glucoside found in the bark of apple trees (Bays 2013). This family of drugs, known as gliflozins, includes several compounds developed for medical use, including canagliflozin, dapagliflozin, empagliflozin, ertugliflozin, ipragliflozin, luseogliflozin, remogliflozin etabonate, sergliflozin etabonate, sotagliflozin and tofogliflozin, and one compound developed recently for use in animals, called velagliflozin.

Mechanism of action

The gliflozins lower blood glucose concentrations by blocking the transport proteins in the kidney that normally facilitate glucose reabsorption. In healthy individuals, ~90% of the glucose that is filtered out of the blood by the kidneys is reabsorbed in the proximal tubule, due to the actions of low-affinity, high-capacity SGLT2 transport proteins (Garcia-Ropero *et al.* 2018). The remaining glucose not reabsorbed by SGLT2, is reabsorbed in the more distal portions of the proximal tubule by SGLT1 proteins, which are high-affinity, low-capacity glucose transporters (Liang *et al.* 2012). Thus, in

the absence of metabolic dysregulation, the excretion of glucose via the kidneys is negligible. Glucosuria only occurs when the capacity of the renal glucose transporters is saturated, and the plasma glucose concentration at which this occurs is known as the renal threshold for glucose excretion. Inhibitors of SGLT2 lower the renal threshold (Rosenwasser *et al.* 2013; Opie 2014). This allows glucose to exit the body via the urine, resulting in glucosuria. In turn, lowering blood glucose concentrations relieves the pressure on the pancreas to secrete insulin, as demonstrated in early research using humans and rodents (List and Whaley 2011; Neumiller 2014). The benefit of lowering insulin concentrations in horses is a reduced risk of laminitis (Meier *et al.* 2018b).

In a recent study, velagliflozin was shown to be effective at reducing the hyperinsulinaemic response of ponies with ID to below the risk threshold for laminitis (Meier *et al.* 2018a). Accordingly, when 12 insulin-dysregulated ponies were pre-treated with the drug for 3 weeks before being challenged with a diet high in non-structural carbohydrates, none developed laminitis, whereas 36% of untreated ponies did develop laminitis on the same diet (Meier *et al.* 2018a). Furthermore, velagliflozin has been shown to be well tolerated in a study of a 16-week duration (Meier *et al.* 2019). This SGLT2 inhibitor is currently undergoing clinical trials and may become the first effective veterinary treatment for ID and the first preventive treatment for laminitis.

Pending the registration of velagliflozin for veterinary use, some veterinarians may be tempted to prescribe other SGLT2 inhibitors off-label. Canagliflozin, sold as Invokana, was the second gliflozin to reach the market, and has been commonly prescribed for humans. Recently, canagliflozin was reported to be effective at decreasing the insulin response to an oral sugar test in a preliminary US study using six horses (Frank 2018). Currently, the potential for adverse effects with canagliflozin use in horses has not been investigated and canagliflozin has been difficult to obtain in Australia since 2016, when it was withdrawn from the Australian Pharmaceutical Benefits Scheme due to its high cost, relative to other compounds in this class.

Many important benefits have been demonstrated through the use of SGLT2 inhibitors in humans with T2DM. These include a reduced risk of heart attack, stroke and death (Thomas and Cherney 2018). Due to loss of glucose, and depending on their dietary intake, subjects treated with gliflozins may also enter negative energy balance and lose weight, or find that their weight stabilises after a period of prolonged weight gain (Thomas and Cherney 2018). This is considered to be a class effect of SGLT2 inhibitors, although it has yet to be demonstrated in horses.

Adverse effects

An obvious theoretical concern about SGLT2 inhibitors is the threat of hypoglycaemia. However, in practice, this is considered a low risk. Whereas the complete blockade of SGLT1 transporters can cause serious effects, chiefly due to their presence in the gut where they facilitate glucose absorption, the effects of SGLT2 inhibitors appear to be more benign. A human genetic disorder that involves a

mutation of the SGLT2 gene, and results in familial renal glucosuria, is regarded as a non-threatening condition in humans (Santer and Calado 2010). Similarly, genetically modified mice that are SGLT2 null also remain healthy (Wright 1998).

It is believed that, in general, SGLT2 inhibitors do not lower the renal threshold for glucose excretion to the point of the hypoglycaemic threshold (Wilding 2014). Additionally, hepatic gluconeogenesis is likely to protect against hypoglycaemia, thus balancing the effect of SGLT2 inhibitors to maintain normoglycaemia (Hoening *et al.* 2018). Finally, no hypoglycaemia was observed in horses treated with velagliflozin for 3 weeks and tested after an overnight fast (Meier *et al.* 2018a).

Another potential risk of lowering blood glucose concentration in horses is hepatic lipidosis. This condition is also influenced by diet, occurring when a severe negative energy balance promotes the mobilisation of fatty acids from adipose tissue, with the end result of hyperlipaemia. This can be life-threatening and may require intensive medical treatment (Foreman 2019). Fortunately, no evidence of this condition was found in ponies during or after 16 weeks of daily velagliflozin treatment, although these animals were well fed (Meier *et al.* 2019). Further studies are needed to accurately assess this risk in horses that are undergoing weight loss due to intense exercise or feed restriction.

Adverse effects of SGLT2 inhibitors in humans include polyuria and polydipsia, constipation, nausea and fatigue, with urinary tract and yeast infections being more common due to glycosuria (Turner *et al.* 2016). None of these effects has been reported in velagliflozin-treated ponies, although only two studies have been reported so far (Meier *et al.* 2019). A more recent concern in humans is that diabetic patients treated with SGLT2 inhibitors experienced a two-fold higher incidence of lower-limb amputation, particularly of the toes, for reasons unknown, but suspected to be associated with dehydration and volume depletion (Neal *et al.* 2017). Although the relevance of this adverse effect to horses is uncertain, it could be a problem in laminitis cases if there is reduced digital perfusion. Another effect observed in humans, that could be of concern for horses, is the negative impact that SGLT2 inhibitors can have on bone mineral density by decreasing the renal reabsorption of phosphate, hence increasing the risk of fractures (Blau and Taylor 2018). This too, remains to be explored in a veterinary context.

Other experimental compounds

GLP-1 inhibitors

The incretin effect (mediated principally by GLP-1 when the meal is dominated by non-structural carbohydrates) is greater in ponies with ID after an oral glucose bolus than in metabolically healthy ponies (de Laat *et al.* 2016). Further, breeds of horses and ponies that are prone to laminitis have greater plasma GLP-1 concentrations after consuming dietary grain (Bamford *et al.* 2015). Thus, although incretins play only a partial role in insulin responsiveness to a carbohydrate-rich meal (de Laat *et al.* 2016), reducing insulin secretion by inhibiting GLP-1 action is a logical therapeutic proposition.

This approach is supported by the fact that equine GLP-1 receptors are present on pancreatic islets, and that the inhibition of equine GLP-1 receptors *in vitro* with synthetic receptor antagonists reduced glucose-dependent insulin secretion (Kheder *et al.* 2018).

A recent pilot study *in vivo* sought to determine the efficacy of a GLP-1 receptor inhibitor in reducing the post-prandial insulin response to a carbohydrate-based meal in ponies. The study found that administration of the GLP-1 receptor inhibitor did reduce insulin responses to grain (Fitzgerald *et al.* 2018). However, only a single dose was tested and the reduction in insulin secretion was modest, and potentially not clinically efficacious. Dose-optimisation studies are required to determine the magnitude of effect at other doses, and the development of equine-specific peptide antagonists may also improve efficacy. However, given that incretins are secondary to glucose in driving insulin secretion in horses, the capacity of GLP-1 inhibitors to attenuate hyperinsulinaemia may always be limited in this species. Despite this, even if the effect is only partial, the potential for this therapeutic avenue to form part of multi-modal therapy in the future remains a possibility.

Sweet taste-receptor inhibitors

Glucose uptake by the small intestine occurs principally via SGLT1, but is boosted by sweet-taste receptors (Tas.R) on intestinal epithelial cells (Nguyen *et al.* 2012). The receptors are also located on intestinal K and L cells, where they function to enhance the incretin effect (Jang *et al.* 2007). Inhibition of these receptors in other species reduced glucose uptake and incretin release, which dampened the insulin response to oral sugar (Sigoillot *et al.* 2012). Although their use has been largely experimental, some compounds are currently used in the management of metabolic dysfunction and T2DM. The dual capacity of Tas.R to target both glucose uptake and incretin release makes them an attractive target for the reduction of equine hyperinsulinaemia.

Previous work determined that Tas.R exist in the equine small intestine (Daly *et al.* 2012), so the use of Tas.R antagonists to reduce hyperinsulinaemia in horses is feasible. A recent study examined two antagonists of the Tas.R *in vivo* for their potential to reduce glucose uptake, incretin release and insulin secretion in ponies eating a carbohydrate-based meal. While one compound did not appear to have any efficacy in horses, the Asian herb *gymnema sylvestris* was able to partially reduce post-prandial hyperinsulinaemia (de Laat *et al.* 2018b). This partial efficacy may be related to the secondary importance of the incretin effect in horses as outlined above or may have been influenced by the purity of the compound used (dried-herb formulation). As Tas.R inhibitors have the potential to inhibit two pathways that lead to the stimulation of insulin secretion, their suitability for the treatment of ID deserves more attention.

Pergolide mesylate

Although not specifically a treatment for laminitis, pergolide has been included in the present review because it is currently one of

only a few registered products for treating an endocrinopathy in horses, in particular an endocrinopathy associated with laminitis. Pituitary *pars intermedia* dysfunction (PPID) is a common condition of older horses and ponies, which could be considered reasonably benign, except for the fact that a subset of cases develop laminitis. The disease is typified by a loss of dopaminergic inhibition of the pituitary axis, specifically of the melanotrophs of the intermediary lobe, which results in increased secretion of pro-opiomelanocortin peptides (McFarlane 2011). Although on the basis of the current understanding of endocrinopathic laminitis pathophysiology it is difficult to reconcile pituitary hyperactivity directly with an increased laminitis risk, the intricate mechanistic details of pituitary dysregulation in horses have not been resolved. It is likely that ID is the instigating factor in PPID-associated laminitis, but the origin of the ID remains obscure. Interactions between insulin and dopamine occur in other species, and whether dopamine insufficiency is a cause, effect or aggravator of the hyperinsulinaemia seen in horses with PPID-associated laminitis, or indeed an innocent bystander, needs to be determined.

Pergolide is an effective treatment for PPID (Donaldson *et al.* 2002). However, whether it has any impact on the incidence or severity of laminitis is uncertain. A recent review concluded that studies examining whether or not pergolide reduces the occurrence or recurrence of laminitis were insufficient in number, and those undertaken were often underpowered or biased (Knowles 2019). Given that some cases of laminitis improved or resolved, some new cases occurred, and some cases recurred during pergolide treatment, the data appear inconclusive and more investigation of this research question is required. With this in mind, pergolide cannot currently be categorised as a treatment for laminitis. In animals with concurrent PPID and ID, other avenues to manage the ID should be pursued in addition to the use of pergolide.

Treating lamellar failure

IGF-1R inhibitors

Several lines of evidence have implicated IGF-1 receptors (IGF-1R) in mediating the effects of insulin on the hoof. These include the fact that insulin receptors are few in the lamellar tissue, whereas IGF-1R are abundant (Burns *et al.* 2013; Nanayakkara *et al.* 2019). At high concentrations, insulin has been shown to bind to IGF-1R in other species, such as humans (Soos and Siddle 1989), rabbits (Nakamura *et al.* 2000), rats (Ding *et al.* 1996) and cattle (Bar and Boes 1984), albeit with very low affinity in the horse (Nanayakkara *et al.* 2019). Cell proliferation, a classical action of IGF-1, is seen in laminitis induced experimentally (de Laat *et al.* 2013a), and in naturally occurring cases (Karikoski *et al.* 2015); IGF-1R are downregulated in hyperinsulinaemic horses *in vivo*, suggestive of IGF-1R activation, despite there being no increase in plasma IGF-1 concentrations (de Laat *et al.* 2013b). Research conducted over the past 4 years, using both explants of lamellar tissue and cultured lamellar cells, has confirmed that insulin does induce cell proliferation and that the peptide activates several downstream second-messengers of IGF-1R (Baskerville

et al. 2018). Thus, while the apparently low binding affinity of insulin for equine IGF-1R remains unexplained, and without being able to rule out a direct effect on insulin or hybrid receptors, the weight of evidence seems to favour at least some involvement of IGF-1R in mediating the effects of insulin.

The development of therapeutic anti-IGF-1R mAb for blocking the effects of IGF-1R activation has had a chequered history, with this approach initially showing great potential for slowing the rate of growth of IGF-1-dependent cancers (Beckwith and Yee 2015). Several major pharmaceutical companies invested in the technology for over a decade, with figitumumab, developed by Pfizer, reaching stage-three clinical trials (Haluska *et al.* 2010). However, none of the prototype antibodies has been adopted for medical use due to issues with long-term efficacy, and the production of figitumumab was discontinued in 2011 (Beckwith and Yee 2015).

The failure of anti-IGF-1R mAb as an anti-cancer treatment has been attributed to its use in unselected patient populations, a lack of biomarkers used to predict the response, and compensatory signalling of other growth factor pathways, particularly insulin and epidermal growth factor (Beckwith and Yee 2015). A singular advantage of its use though, is that this class of agents appears to cause very few adverse effects compared with other anti-cancer treatments (Beckwith and Yee 2015). The periodic use of anti-IGF-1R mAb in horses, to alleviate the effects of hyperinsulinaemia on the hoof during brief periods of hyperinsulinaemia, or to limit the damage caused by insulin following an acute episode of laminitis, is currently being investigated and could provide a safe and effective complement to other anti-laminitis strategies.

The reason that anti-IGF-1 mAb was developed in the first place, was the difficulty experienced in trying to synthesise small-molecule inhibitors that are selective for the IGF-1R. This is partly due to its close homology between IGF-1R and insulin receptors, and the existence of hybrid insulin and IGF-1R. Nevertheless, a few such drugs do exist, which, rather than blocking the binding of IGF-1, inhibit the tyrosine kinase second messenger pathway (Blum *et al.* 2000). An examination of inhibitors of tyrosine kinases may be another future avenue for the development of drugs for endocrinopathic laminitis.

MMP inhibitors

Matrix metalloproteinases (MMPs) are enzymes that break down connective tissue. While these proteins may be involved in the normal remodelling of lamellar tissue, it has been demonstrated that lamellar explants maintained in culture medium break down when exposed to the MMP activator aminophenylmercuric acid, and that this effect can be prevented by the MMP inhibitor batimastat (Pollitt *et al.* 1998). The expression of MMPs is increased naturally in many inflammatory conditions, including during the developmental phase of experimental, sepsis-related laminitis (Kyaw-Tanner and Pollitt 2004). The fact that MMPs can also be activated by certain bacteria led to the proposition that inflammatory forms of laminitis may be caused as a result of the leakage of bacteria or bacterial

endotoxins from the gut, specifically *Streptococcus bovis*, following carbohydrate overload. However, although some markers of inflammation are evident in endocrinopathic laminitis, these are generally seen late in the onset of the disease, and are thought to be a symptom, rather than a cause of tissue damage (de Laat *et al.* 2011). Furthermore, because laminitis can be induced experimentally by the intravenous infusion of insulin (Asplin *et al.* 2007; de Laat *et al.* 2010), there is no need to invoke the actions of bacterial toxins, or the activation of MMP, to explain the pathogenesis of this form of the disease. Accordingly, the value of MMP inhibitors in treating endocrinopathic laminitis remains in question.

Vasoactive amines

In the carbohydrate overload model of laminitis, it has been argued that the rapid fermentation of carbohydrates by lactobacilli and *S. bovis*, in particular, leads to the production and leakage from the hind-gut of vasoactive amines (Bailey *et al.* 2003). These amines are thought to cause the displacement of 5-hydroxytryptamine from platelets, which, in turn, restricts blood flow to the hoof and causes laminitis (Bailey *et al.* 2004). Evidence to support this theory includes the observation that tryptamine phenylethylamine infusions do cause a significant decrease in digital arterial blood flow, accompanied by a decrease in the hoof wall surface temperature (Bailey *et al.* 2004). Furthermore, certain studies have demonstrated vascular dysfunction, both systemically in horses with PPID (Keen *et al.* 2004) and in the hoof in horses with endocrinopathic laminitis (Morgan *et al.* 2016).

The hypoperfusion theory of laminitis once excited significant interest among equine researchers and, following its introduction, several approaches to improve blood flow to the hoof and treat laminitis were suggested. Pharmacologic approaches that have been used experimentally include the use of agents that prevent platelet activation, or improve red blood cell deformability (Weiss *et al.* 1998; Hood *et al.* 2001), endothelin antagonists (Eades *et al.* 2007), nitric oxide (Berhane *et al.* 2008) and glycerol trinitrate (Hoff *et al.* 2002).

However, Pollitt has argued strongly against the hypoperfusion theory, stating that laminitis does not occur if the foot is in a state of vasoconstriction during the developmental phase (Pollitt 2004). This theory is also difficult to reconcile with the efficacy of cryotherapy as a treatment for the condition (van Eps *et al.* 2004). Instead, Pollitt argued that laminitis development coincides with an increase in sublamellar blood flow (Pollitt and Davies 1998), and this is certainly consistent with observations of an increase in hoof wall surface temperature during the developmental phase of laminitis in horses infused with insulin (de Laat *et al.* 2012). Thus, despite early interest in the hypoperfusion theory and the role of vasoactive amines, there has been little progress in this field over the past decade, when the focus of endocrinopathic laminitis research began to switch to the study of insulin and its actions.

Although not technically a 'pharmacologic treatment option' for lamellar failure, distal limb cryotherapy is used for the prevention and treatment of laminitis. Its use has been

more widespread in inflammatory forms of laminitis, where prediction of laminitis onset is easier than for endocrinopathic laminitis, where disease onset can be latent and insidious. Cooling of the limb can achieve a reduction in the severity of the lesion in acute disease (van Eps *et al.* 2004), and research is continuing into the timelines, temperatures and types of cooling that can achieve the best outcomes (van Eps and Orsini 2016; Morgan *et al.* 2018), while minimising pathologies associated with prolonged cooling (Proctor-Brown *et al.* 2018).

Treating pain

Although the administration of analgesics does not target disease pathophysiology, it is paramount to laminitis management from a welfare perspective. The choice of drug will depend on the pain scale, disease chronicity, the animal's circumstances and the owner's input. Optimal pain-management regimes are usually possible only in an intensive hospital-type setting, where ambulation can be minimised and constant-rate intravenous administration of medications is possible. In the field, routes of administration are likely to be more limited, and the choice of drug is restricted accordingly. A key concern when choosing a pain-relief strategy for laminitic patients is ensuring that pain relief does not inadvertently worsen disease by promoting excessive movement and further lamellar destabilisation. The goal of pain relief should be to achieve comfort, without compromising treatment success.

Anti-inflammatories and paracetamol

Non-steroidal anti-inflammatory drugs (NSAIDs) mediate pain relief through both central and regional effects, whereas paracetamol (acetaminophen) mainly acts centrally, with similar analgesic effects, but minimal anti-inflammatory effects. Several NSAID options are marketed for analgesia in horses, although paracetamol use is off-label in this species. Both NSAIDs and paracetamol are suitable for use in field-based settings and NSAIDs are the mainstay of laminitis pain therapy. These are affordable drugs, and doses or dosing schedules can be manipulated to some extent to individualise the approach.

A decrease in the production of inflammatory mediators, such as prostaglandins and eicosanoids, is achieved by NSAIDs through the inhibition of the cyclooxygenase (COX) enzymes (1 and 2; Beretta *et al.* 2005). Some drugs in the class are non-selective, for example, phenylbutazone, and act to inhibit both COX-1 and COX-2. Phenylbutazone has been the mainstay of laminitis analgesia for laminitic patients for many years and continues to be used widely. Flunixin, firocoxib and meloxicam are other readily available options (Duz *et al.* 2019).

In recent years, COX-2 selective NSAIDs have been favoured in other species, due to their proposed lower risk of gastrointestinal ulceration secondary to reduced activity through the COX-1 pathway (COX-1 is widely distributed in the body). In addition, it has been suggested that they are more specific for relieving pain, due to the inducible nature of COX-2 at sites of inflammation (Everts *et al.* 2000).

However, more recently there have been concerns over adverse effects associated with their use, particularly in humans, and some have been withdrawn from the market (Sibbald 2004). While *in vitro* data have confirmed the COX-2 selectivity of meloxicam and carprofen in horses (Beretta *et al.* 2005), prescription of these COX-2 selective drugs, although increasing, remains less frequent than that of phenylbutazone (Duz *et al.* 2019). In fact, one study has reported that non-selective NSAIDs provide better pain relief for laminitis than the newer selective (or preferential) options, such as meloxicam (Banse and Cribb 2017). However, overall, there are few and conflicting data on whether one NSAID drug provides analgesia superior to that provided by any other NSAID drug in horses (Duz *et al.* 2019).

The administration of NSAIDs is usually via the oral route, which facilitates the treatment of field-based cases. However, responses to NSAID treatment can be variable, with many factors likely to be contributing to inadequate analgesia (Schatzmann *et al.* 1990). Difficulties with dosing schedules and drug administration, coupled with inter- and intra-animal variations in responses to drugs and poor administrator compliance, can all contribute to NSAID inefficacy. In many cases of laminitis, pain relief may be required for a prolonged duration, and a reduced compliance by the animal may also contribute to treatment failure. The use of ongoing pain control and long-term NSAID therapy also increases the risk of adverse drug reactions. All NSAIDs are associated with an increased risk of gastric ulceration (Everts *et al.* 2000).

Paracetamol inhibits endocannabinoid re-uptake in the brain to induce analgesia via central pathways. However, despite its widespread use in human medicine, the mechanism of action of this analgesic is not well understood, and there are likely to be other, more poorly defined, mechanisms of paracetamol action. Little information is available on paracetamol efficacy and safety in horses. While there are few adverse effects associated with the use of paracetamol in humans, severe adverse reactions do occur in other domestic animal species (e.g. cats) due to differences in drug metabolism (Savides *et al.* 1984). A comparative study using domestic animal species showed that paracetamol is highly protein-bound in horses, which delays drug clearance, and it is also highly bioavailable compared with dogs (Neirinckx *et al.* 2010). Further, a recent study determined that the drug half-life is significantly extended following multi-dose treatment regimens (Mercer *et al.* 2020). Thus, it should not be assumed that paracetamol pharmacokinetics can be extrapolated from other species, and further study in horses is warranted. However, the limited data available suggest that paracetamol may be a safe and effective analgesic for laminitis pain (West *et al.* 2011; Mercer *et al.* 2020).

Other analgesic agents

Despite heavy reliance on NSAIDs for laminitis pain management, other analgesic drugs are increasingly being used. Unfortunately, many of these medications are yet to be adequately researched in horses, and there is little published

data on how to use them safely and efficaciously. Gabapentin is one such drug, despite minimal data to support its use (Sanchez and Robertson 2014). Gabapentin inhibits signalling through calcium channels that are upregulated in neuropathic pain states, thereby modulating the perception of pain in the central nervous system (Jones *et al.* 2007; Colloca *et al.* 2017). An advantage of gabapentin is that it can be administered orally. This facilitates its use in non-hospital-based cases, although poor oral bioavailability reduces drug efficacy (Terry *et al.* 2010). The use of gabapentin for managing pain associated with laminitis needs to be supported by more studies that demonstrate its safety and efficacy, especially when combined with other drugs in a multi-analgesic approach.

As with gabapentin, use of the centrally acting analgesic tramadol (an atypical, weak opioid) for managing pain in cases of laminitis is increasing, despite there being limited data on its safety profile in horses. A small study found that oral tramadol was effective at 10 mg/kg bodyweight in four horses with laminitis, while a dose of 5 mg/kg bodyweight was not (Guedes *et al.* 2016). The low oral bioavailability of tramadol is likely to limit its effectiveness at lower dose rates (Shilo *et al.* 2008). Another study found intravenous tramadol to be ineffective when used as a single therapy, but efficacious when administered in combination with paracetamol (Tavanaeimanesh *et al.* 2018). The short-term nature of this, and other studies on tramadol use in horses, prevent adequate analysis of the likelihood of adverse effects occurring with the use of tramadol in treating chronic pain. Given that the full spectrum of the mechanisms of action of tramadol is not fully elucidated in any species (Bravo *et al.* 2017), and the fact that opioid drugs are associated with a risk of reduced gastrointestinal motility, caution with prolonged use of tramadol in horses is needed, especially until further data are published.

For hospitalised cases of laminitis, the option of using analgesics that can be administered only intravenously or via regional perfusion (e.g. epidural administration for hindlimb pain) exists. True opioids, such as fentanyl (as patches), morphine or butorphanol, can be used to help manage refractive pain, but their administration needs to be carefully monitored due to the risk of adverse neurological and gastrointestinal effects (Levionnois *et al.* 2018). A constant-rate infusion of the N-methyl-D-aspartate receptor antagonist ketamine hydrochloride has also been used to help manage laminitis pain (Guedes *et al.* 2012). Although ketamine is an anaesthetic agent, low-dose ketamine infusion has been shown to provide effective analgesia, especially when co-administered with other analgesics, such as tramadol (Guedes *et al.* 2012).

Another short-acting compound that has been suggested for laminitis treatment is lignocaine. The short half-life of this local anaesthetic also necessitates a constant-rate infusion. Lignocaine blocks sodium channels in nerves, although the mechanism of action when infused intravenously is uncertain. While there is limited evidence on the safety of using lignocaine intravenously in horses, and there is a known risk of neurological and cardiac toxicity in this, and other species (Vieitez *et al.* 2017; Masic *et al.* 2018), a limited

number of studies identified few adverse effects at the dose rates tested (Malone *et al.* 2006; Risberg *et al.* 2014).

Overall, infusions of opioids, lignocaine, ketamine or combinations of these drugs, appear to be used most often as part of a multi-modal analgesic approach for hospitalised patients with intractable pain (Sanchez and Robertson 2014). These agents may have a useful role in managing severe cases of laminitis, but the use of such drug combinations requires careful planning and patient monitoring, and far more scientific evidence is needed to support their application.

Conclusions

While the pharmacologic treatment of laminitis is currently limited to the management of pain, several novel medications that aim to target other facets of the disease are currently being developed and may become available to the clinician in due course. These drugs will need to be incorporated into a multi-faceted approach to laminitis therapy, which addresses required lifestyle changes, particularly in obese equids, the foot pathology, pain and the underlying endocrinopathic disturbance. A significant cause for optimism in the quest for new therapies to prevent or treat laminitis is the availability and emergence of new therapies for allied conditions in humans. For example, the increased prevalence of T2DM in humans has led to the development of a raft of new therapies that could be applied in equine metabolic syndrome, lessening the risk of laminitis. Similarly, in terms of treating the foot, the development of therapeutic antibodies owes much to human cancer research. Ironically, while some of these treatments have failed to become commercial realities in human medicine, due to adverse effects or a lack of long-term efficacy, these issues may not necessarily be a problem in horses, and this species could be the first to reap the benefits of these important medical advances.

Conflicts of interest

The authors declare no conflicts of interest.

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