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Persistent digital hyperthermia over a 48 h period does not induce laminitis in horses

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ABSTRACT

Persistent digital hyperthermia, presumably due to vasodilation, occurs during the developmental and acute stages of insulin-induced laminitis. The objectives of this study were to determine if persistent digital hyperthermia is the principal pathogenic mechanism responsible for the development of laminitis.

The potent vasodilator, ATP–$MgCl_2$ was infused continuously into the distal phalanx of the left forefoot of six Standardbred racehorses for 48 h via intra-osseous infusion to promote persistent digital hyperthermia. The right forefoot was infused with saline solution and acted as an internal control. Clinical signs of lameness at the walk were not detected at 0 h, 24 h or 48 h post-infusion. Mean ± SE hoof wall temperatures of the left forefoot (29.4 ± 0.25 °C) were higher ($P < 0.05$) than those on the right (27.5 ± 0.38 °C). Serum insulin (15.0 ± 2.89 μIU/mL) and blood glucose (5.4 ± 0.22 mM) concentrations remained unchanged during the experiment. Histopathological evidence of laminitis was not detected in any horse.

The results demonstrated that digital vasodilation up to 30 °C for a period of 48 h does not trigger laminitis in the absence of hyperinsulinaemia. Thus, although digital hyperthermia may play a role in the pathogenesis of laminitis, it is not the sole mechanism involved.

Introduction

Laminitis is an important disease of the horse, characterised by lameness, an increased digital pulse and palpably warm hooves (USDA, 2000). While it is assumed that increased blood perfusion occurs during the acute phase of the disease, there has been considerable debate as to the role of the vasculature during disease development. While some evidence suggests decreased blood flow to the foot occurs (Adair et al., 2000), or that blood is shunted away from the digital microcirculation through dilated arteriovenous anastomoses (Robinson, 1990), other studies point to the raised hoof temperature (and presumably increased blood flow to the foot) that occurs during the developmental phase of both carbohydrate (Pollitt and Davies, 1998) and insulin (de Laat et al., 2010) induced laminitis. Insulin causes vasodilation through its interaction with vascular endothelium and the release of nitric oxide (Baron, 1996). This is a potential mechanism for the digital vasodilation seen during hyperinsulinaemic laminitis in horses (de Laat et al., 2010).

As the developmental phase of naturally-occurring laminitis often goes unrecognised, the opportunity to monitor blood flow during this pre-clinical phase is missed. In particular, there is little opportunity to study the development of endocrinopathic laminitis, as the precise time of onset in at-risk individuals can be difficult to predict (Geor, 2008; Menzies-Gow et al., 2010). Experimentally-induced hyperinsulinaemic laminitis is associated with persistently elevated hoof wall temperatures during disease development that are consistently higher, and less variable than the hoof temperatures recorded in controls (de Laat et al., 2010). The increased hoof temperature recorded in that study was considered likely to be a reflection of increased digital perfusion and was attributed to the vasodilatory effects of insulin.

The purpose of the current study was to investigate the role of persistent digital vasodilation in the pathogenesis of insulin-induced laminitis. Persistent digital vasodilation was induced in horses over 48 h with a combination of adenosine 5′ triphosphate (ATP) and magnesium chloride ($MgCl_2$), in order to determine if digital hyperthermia *per se* is sufficient to cause laminitis in the absence of hyperinsulinaemia.

ATP is a powerful vasodilator and acts via purinoceptors located on vascular endothelial cells (Burnstock and Kennedy, 1986). The use of a combination of ATP and $MgCl_2$ has been investigated as a potential therapy to improve perfusion following sepsis (Chaudry et al., 1980), shock (Chaudry et al., 1976) and ischaemia (Paskitti and Reid, 2002). The combination is safe for IV use in horses and increases blood flow in a dose-dependent manner up to a maximal safe

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infusion rate (Tetens et al., 1999). The combination of ATP–$MgCl_2$ was selected for use in the current study as it was likely to increase digital blood flow following intra-osseous infusion into the distal phalanx. A secondary aim was to determine the suitability of ATP–$MgCl_2$ as a vasodilatory agent following intra-osseous perfusion.

Intra-osseous infusion techniques have been used successfully in the metacarpal (Mattson et al., 2004; Keys et al., 2006) and phalangeal (Rubio-Martinez et al., 2005; Nourian et al., 2010) bones of horses. There are numerous medullary sinuses in the body of the distal phalanx of the horse that are traversed by abundant blood vessels that connect directly to the lamellar vasculature via many anastomoses (Nourian, 2009). Intra-osseous infusion of the distal phalanx (IOIDP) provides a means of directly administering substances into the terminal digital circulation and lamellar microenvironment (Nourian et al., 2010). In our study, continuous IOIDP was preferred to intra-arterial or IV catheterisation due to the extended nature of the experiment (48 h) and the perceived likelihood of equipment failure during prolonged catheterisation of both forelimbs in a standing, conscious horse. Furthermore, by using this method successful infusion can be achieved without the need for a tourniquet (Nourian et al., 2010). We hypothesised that the prolonged infusion of ATP–$MgCl_2$ into a forelimb would result in lamellar vasodilation and persistent hyperthermia, but not the development of laminitis.

Materials and methods

Animal selection

Six clinically healthy (mean bodyweight, 430 ± 17 kg), male Standardbred racehorses of moderate body condition (body condition score, 4-5/9) (Henneke et al., 1983) were randomly selected. The animals had a mean age of 5.2 ± 0.73 years and did not exhibit evidence of endocrine disease or insulin resistance (cresty neck score, 0/5) (Carter et al., 2009a) on clinical examination. All horses were videotaped while being walked and 'trotted out', before, during and after the experiment, and any unsound animals were excluded at study commencement. Plain, lateral radiographs were taken, and visual inspection of both front feet was performed, before the study to identify horses with pre-existing foot pathology.

The horses were paired randomly and the experiment was conducted in a purpose-built, climate-controlled facility as three replicates over a 10 day period during sub-tropical mid-winter. The horses were accommodated in the facility for 48 h prior to study commencement to facilitate environmental acclimatisation. Appetite, demeanour, heart and respiratory rate and rectal temperature were monitored every 4 h throughout the experiment. Urinalysis was performed prior to, and at the end of the study to assess urine specific gravity and glucose, ketone, protein, and both red and white blood cell content. Medium quality lucerne chaff, lucerne hay and water were available to the horses ad libitum from 48 h preceding the start up to the termination of the study.

The experimental protocol was approved by the Animal Ethics Committee of The University of Queensland (authorisation number SVS/108/09/RIRDC) in compliance with the Animal Welfare Act of Queensland (2001) and The Australian Code of Practice for the Care and Use of Animals for Scientific Purposes (2004). All horses were subject to continuous veterinary monitoring throughout the experiment.

Blood sampling

Blood samples (20 mL) were taken at the beginning and end of the study via a 14 G IV, extended-use catheter (MilaCath, Mila) placed in the left jugular vein, to monitor standard haematological (vacutainer containing EDTA, Greiner) and biochemical (plain vacutainer, Greiner) parameters. Blood samples (5 mL) were also drawn to measure serum insulin and blood glucose concentrations at 6 h intervals throughout the study. Blood glucose was measured immediately using a handheld glucometer (Accucheck Go, Roche) previously calibrated against the hexokinase method for equine blood ($\rho_c = 0.96$). Serum was obtained by allowing the sample to clot for 30 min and centrifuging at 3000 *g* for 10 min. Aliquots of serum (1 mL) were stored at −80 °C until analysed. Serum insulin concentrations were determined for each sample using a radioimmunoassay (Coat-a-count, Siemens) previously validated for use in horses (McGowan et al., 2008).

Formulation and administration of ATP–$MgCl_2$ solution

The ATP–$MgCl_2$ solution was prepared using adenosine 5′-triphosphate disodium salt (A3377, Sigma) and magnesium chloride hexahydrate (M2670, Sigma) as previously described (Chaudry, 1982). Adenosine 5′ triphosphate (0.605 g, 1000 μmol) of was dissolved in 2.5 mL of cold, de-ionised water and the pH adjusted to 7.0 using 1.0 M sodium hydroxide (NaOH). The final pH of the solution was increased to 7.4 with 0.1 M NaOH. The volume was made up to 5 mL with cold, de-ionised water and kept on ice. Magnesium chloride (0.2033 g, 1000 μmol) was dissolved in 5 mL of cold, de-ionised water, mixed with the ATP solution and sterilised through a 0.22 μm filter (Millipore). This solution was then kept on ice until used or was stored at −20 °C in 10 mL aliquots for up to 2 weeks.

Although the maximal safe IV infusion rate of ATP–$MgCl_2$ in horses is 0.3 mg/kg/min, a dose rate of 0.2 mg/kg/min is sufficient to achieve peripheral vasodilation without accompanying hypotension (Tetens et al., 1999). For the current study, a dose rate of 0.2 mg/kg/min was selected for intra-osseous infusion and was calculated based on the weight of the hoof rather than the horse. The average weight (1.61 ± 0.05 kg) of the distal limb of a Standardbred horse was calculated by weighing 12 fresh cadaver limbs disarticulated at the fetlock obtained from a local abattoir.

The ATP–$MgCl_2$ solution (7.66 mL, 463.68 mg) was mixed with 22.34 mL of cold 0.9% saline (Baxter) and infused into the left forefoot over 24 h via a 30 mL, reusable, spring-driven syringe pump (Springfusor, Go Medical) through flow control tubing (Springfusor, Go Medical), so that a constant infusion rate of 0.02 mL/min was achieved. The springfusor was reloaded with fresh solution and the flow control tubing was re-attached for the second 24 h period of the study. The right forefoot was infused with 30 mL of cold 0.9% normal saline over each 24 h period using an identical system.

Intra-osseous infusion technique

The horses were sedated using 0.04 mg/kg of IV romifidine hydrochloride (Sedivet, Boehringer Ingelheim) prior to the administration of bilateral, abaxial, perineural, digital nerve blockade using 2% lignocaine (Troy). An insertion site was marked on the dorsal surfaces of the front hooves, 25 mm below the hairline and 30 mm lateral to the midline, prior to the hooves being cleaned with a wire brush and disinfected with chlorhexidine. A circular area of 10 mm radius around the insertion point was then cauterised to a depth of 2 mm using a custom-made iron, heated to 'red-hot' in a gas forge.

An intra-osseous needle (EZ IO AD 25 mm, Vidacare) was inserted at right angles to the hoof wall at the midpoint of the sterilised area using a specialised drill (EZ IO Power Driver, Vidacare). The needle was passed through the hoof wall and lamellar region into the dorsal cortex of the distal phalanx (Fig. 1). Once resistance to drilling decreased, indicating penetration of the dorsal cortex, the drill and needle stylet were removed and a pre-loaded syringe of heparinised saline with 15 cm long extension tubing (EZ Connect, Vidacare) was attached to the luer-lock hub of the intra-osseous needle, and the system flushed. Once patent, the Springfusor was attached to the needle via the flow control tubing, and the infusion commenced. The needle was protected with Elastoplast (Beiersdorf) and the Springfusor was secured in a custom-designed neoprene boot (Fig. 2). Further local anaesthesia was not required. The horses were fitted with equine nappies (Equisan) to avoid urine or faecal contamination of the infusion site.

Measurement of hoof wall surface and ambient temperature

A surface thermistor (TinyTag, Gemini) was placed on the midline of the dorsal aspect of the hoof wall, 25 mm below the hairline and 30 mm medial to the midline, on both front feet of each horse (Fig. 2). The hoof wall surface temperature (HWST) was measured separately for both front feet every 5 min throughout the experimental period by a data logger connected to the thermistor. Ambient temperature was measured every 5 min by an identical thermistor and data logger placed within 1 m of the horse.

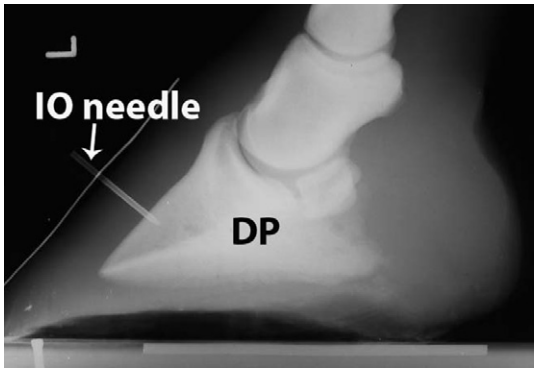

Fig. 1. Plain lateral radiograph of the left distal limb of a horse following placement of the intra-osseous (IO) needle through the hoof wall and lamellae with its tip inserted into the dorsal cortex of the distal phalanx (DP).