Effect of lithium chloride on inflammatory processes in the adult horse:
ex vitro PAMP-induced cytokine responses in whole blood culture

Jonathan Tomkovitch, Elizabeth Schroepfer, Mindy Wolfe, Charles Wiedmeyer, Alex Bukoski, Amy DeClue, Tim Evans, & Philip Johnson. College of Veterinary Medicine, University of Missouri, Columbia, MO.

Abstract

Laminitis is a common and potentially devastating disease of the equine hoof for which effective preventive and therapeutic strategies are needed. Laminitis occurs following activation of the innate immune system and the degradation of basal epithelial cell attachments to both adjacent cells and to the underlying basement membrane. Recently, we showed that reduced expression of β-catenin and integrin-β4 in hoof lamellar basal epidermal cells is a component of laminitis resulting from activated innate inflammation. This finding may explain diminished cell-to-cell and cell-to-basement membrane attachment, and is possibly a consequence of suppressed canonical Wnt signaling pathways. Lithium chloride (LiCl) both supports Wnt signaling and inhibits innate inflammatory responses. Therefore, LiCl administration might prevent laminitis through support of canonical Wnt signaling pathways and inhibition of innate immune responses. As a first step toward employing LiCl for prevention of laminitis, we investigated the effect of systemically-administered LiCl on Pathogen-Associated Molecular Pattern (PAMP) motif-induced cytokine secretion in cultivated whole blood (Cwb) ex vitro. Blood was obtained from 8 healthy, adult horses before (time 0), during (+2 h), and at the conclusion of a 24-hour LiCl treatment at a titrated dose intended to maintain a steady state plasma Li concentration in the 0.8-1.2 mM therapeutic range. In order to ensure that the circulating Li concentration remained in the therapeutic range throughout the 24-hour treatment period, plasma Li concentration was measured every 4 h as a basis for adjustment of LiCl dose. PAMP-stimulated cytokine (IL-1 and TNF) production in Cwb was determined using methods developed in our laboratory.

Background

Laminitis is a debilitating disease affecting horses, owners, and the equine industry. Each year, horses succumb to this disease and many are compromised for life. Laminitis is characterized by separation of the epidermis from the dermis in the equine hoof.[1] Due to this separation, the third phalanx rotates and sinks within the hoof, causing severe lameness.[1] Therefore, it is important that possible treatments and preventive strategies for this disease are diligently pursued.

Diminishment of the canonical wnt signaling pathway in hoof epidermal cells has recently been shown to occur as a component of the laminitic process associated with activated innate imflammation.[1] This is important because proper function of this pathway is essential for normal β-catenin and integrin-β4 expression, two vital components of basal epidermal cell attachment (Figure 1).[1] β-catenin is a component of adherens junctions between cells and integrin-β4 is a component of hemidesmosomes, which form the attachments between the basal epidermal cells and the basement membrane. (Figure 2)[1] Loss of these attachments, critical for the complex interwoven dermo-epidermal lamellar interface of the hoof (Figures 3, 4, 5), occurs during the early stages of laminitis.[1] Therefore, is has been suggested that wnt signal pathway failure in inflammatory laminitis may be significantly responsible for the observed loss of basal epidermal cell attachments.[1] Lithium chloride is an effective stimulator of the wnt pathway and may also reduce innate immune responsiveness. Therefore, treatment of at-risk horses with lithium chloride represents a potentially attractive preventive strategy for laminitis.

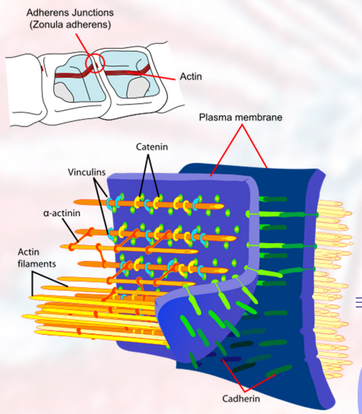

Figure 1-Schematic of adherens junctions

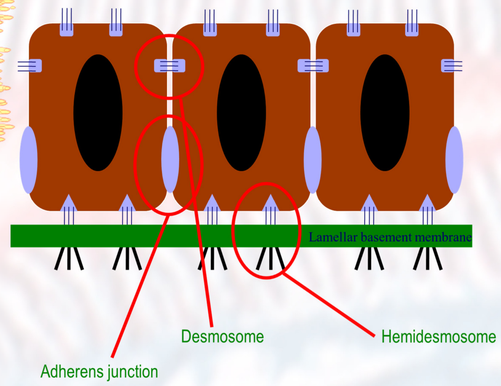

Figure 2- Schematic of the basal epidermal cell/basement membrane interface

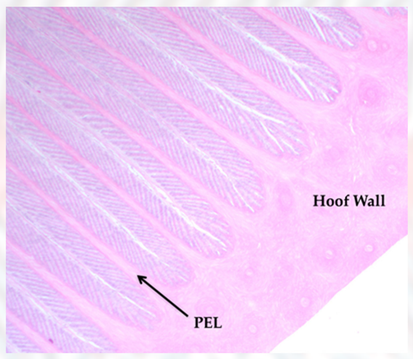

Figure 3- Low powered microscopic appearance of the normal equine hoof lamellar interface. PEL-primary epidermal lamella. Stain – H&E routine

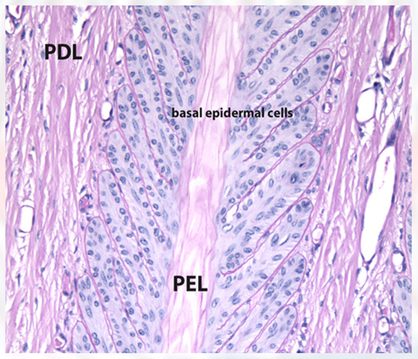

Figure 4- Higher magnification of the equine hoof lamellar interface. PEL-primary epidermal lamella, PDL-primary dermal lamella. Stain – PAS (shows basement membrane stained as magenta-colored line)

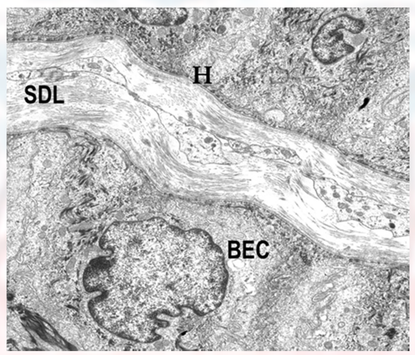

Figure 5- Ultrastructural appearance of the equine hoof lamellar interface at the secondary dermal/epidermal interface. Note hemidesmosomes (H) against basement membrane. BEC-basal epidermal cell, SDL-secondary dermal lamella.

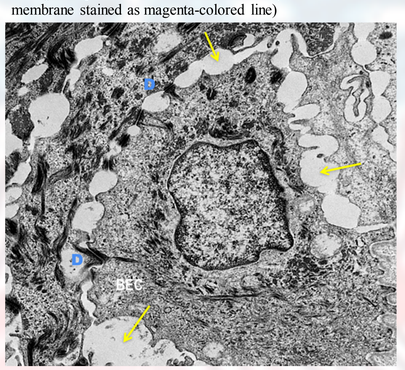

Figure 6-Ultrastructural appearance of basal epidermal cell with diminished cell-cell and cell-basement membrane attachment during laminitis. Note the dilated intercellular spaces (arrows) – resulting from loss of adherens junction. Desmosomal cell-to-cell attachments (D) appear to be preserved at this stage.

Hypothesis

Hypothesis- Treatment of normal adult horses using lithium chloride will decrease the secretion of interleukin-1β (IL-1β) and tumor necrosis factor (TNF) when stimulated by Pathogen Associated Molecular Patterns (PAMPS), such as lipopolysaccharide (LPS), peptidoglycan (PG), and lipoteichoic acid (LTA) in a whole blood culture (Cwb) system, *ex vitro*.

Animals

Eight adult horses were determined to be healthy based on physical examinations and their use in this experiment was approved by the IACUC. Catheters were placed in both jugular veins with the left used to obtain blood for measurement of plasma lithium concentrations and whole blood culture, and the right jugular vein was used for lithium chloride infusion.

Methods

In order to maintain plasma lithium concentration in the therapeutic range, one horse was administered a bolus of 8.25 ml of lithium chloride and then 120 mmol of lithium chloride was given via a slow-drip IV in 3 liters of deionized, distilled water over a course of 24 hours. A second horse was then administered a bolus of 8.25 ml of lithium chloride but the amount used with the deionized water was increased to 360 mmol over a 12 hour period. Physical exams were performed at each time point to note any effects of lithium infusion.

Whole Blood Culture- Using aseptic technique, 2.5 ml of blood was obtained from each horse and within 30 minutes was mixed with 2.5 ml of Cwb culture medium and placed in a water bath for 15 minutes. A sample of each mixture (900 µl) was placed into 12-well plates and 100 µl of PAMPS (LPS, LTA, and PG) were then added into their respective wells, with PBS serving as the control (Figure 7). The plate was then mixed using a rocker for 5 minutes and then incubated for 24 hours at 37 °C. After incubation, the supernatant in each well was extracted and placed in Eppendorf Tubes. The tubes were then centrifuged for 15 minutes. Supernatant was then extracted from these tubes and placed into additional Eppendorf Tubes which were frozen to -80 °C for cytokine analysis. PAMP-stimulated cytokine production (TNF and IL-1β) was determined using methods developed in our laboratory.

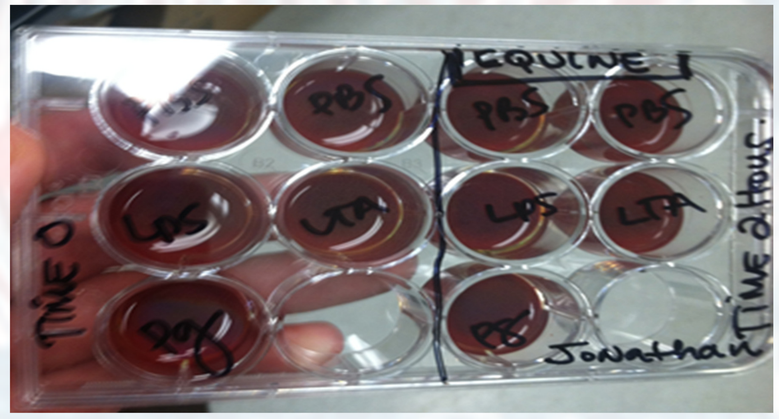

Figure 7- Whole blood culture plate

Preliminary Results

- Infusion of lithium did not cause any untoward physical responses
- Preliminary results indicate that our lithium chloride IV infusion is working to successfully maintain plasma lithium concentrations throughout the 24 hour period, but we have not yet attained a satisfactory therapeutic target. (Figure 8, 9).

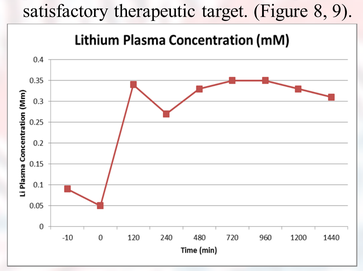

Figure 8- Plasma lithium concentrations over a 24 hour period

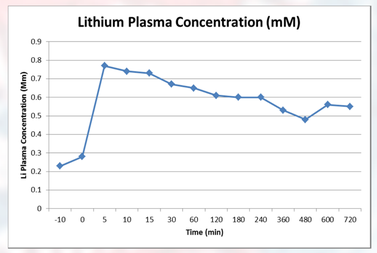

Figure 9-Plasma lithium concentrations over a 12 hour period

- Analysis of PAMP-induced cytokine (TNF and IL-1β) release is currently in progress (results pending).

References

1. Wang L, Pawlak EA, Johnson PJ, Belknap JK, Eades S, et al. (2013) Impact of Laminitis on the Canonical Wnt Signaling Pathway in Basal Epithelial Cells of the Equine Digital Laminae. PLoS ONE 8(2): e56025. doi:10.1371/journal.pone.0056025

Acknowledgements

Funding was provided by the Animal Health Foundation of St. Louis and the University of Missouri College of Veterinary Medicine