Effect of lithium chloride on inflammatory processes in the adult horse: neutrophil phagocytosis and oxidative burst capacity assessed using flow cytometry

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Abstract

Laminitis is a devastating disease of the equine hoof for which effective preventive and therapeutic strategies are currently lacking. Laminitis occurs following activation of the innate immune system and the degradation of the hoof's basal epithelial cell attachments to both adjacent cells and to the underlying basement membrane. Recently, it has been shown that reduced expression of β-catenin and integrin-β4 in hoof lamellar basal epithelial 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 neutrophil function (a proxy for innate immune responsiveness). Blood was obtained from 8 healthy, adult horses before (time 0), during (+2 h), and at the conclusion of a 24-hour treatment period with LiCl. A titrated dose intended to maintain a steady state plasma Li concentration in the 0.8-1.2 mM therapeutic range was used. 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. Neutrophils (phagocytic capacity and oxidative burst capacity) were assessed via flow cytometry.

Background

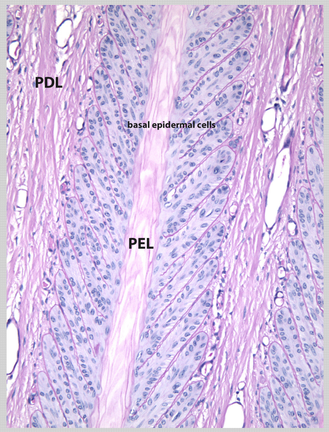


Figure 1

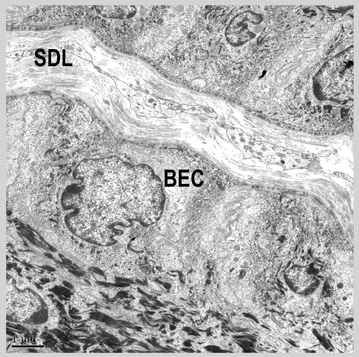


Figure 2

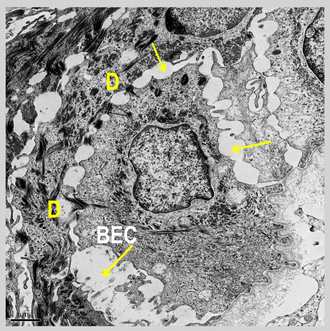
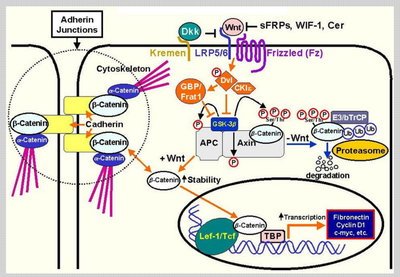


Figure 3



Howard JC, et al. (2003)

Figure 4

Figure 1-Low powered microscopic appearance of the equine hoof lamellar interface; PAS stain
Figure 2-Ultrastructural appearance of the equine hoof lamellar interface at the secondary dermal/epidermal interface
Figure 3-Ultrastructural appearance of basal epidermal cell with diminished cell-cell and cell-basement membrane attachment
Figure 4-Canonical Wnt signaling pathway

Hypothesis and Objectives

Hypothesis-
Lithium chloride treatment inhibits neutrophil function in horses.

Objectives-
- Identify a cohort of healthy adult horses for treatment using either IV saline (control) or IV lithium chloride (LiCl).
- Obtain blood at specific time points to measure plasma lithium concentrations, neutrophil phagocytosis, and oxidative burst functions

Methods

Blood will be collected from 6-8 healthy adult horses to be analyzed via flow cytometry for neutrophil phagocytosis and oxidative burst capacity. Each horse will receive an initial bolus of LiCl to achieve the therapeutic plasma concentration of 0.8-1.0mmol/L via a 16 gauge catheter placed in the right jugular vein. LiCl will then continuously be infused over 24 hours at a concentration that will maintain this therapeutic concentration of 0.8-1.0 mmol/L. Blood samples will be collected via a 14 gauge catheter placed in the left jugular vein at 0, +2, and +24 hours.

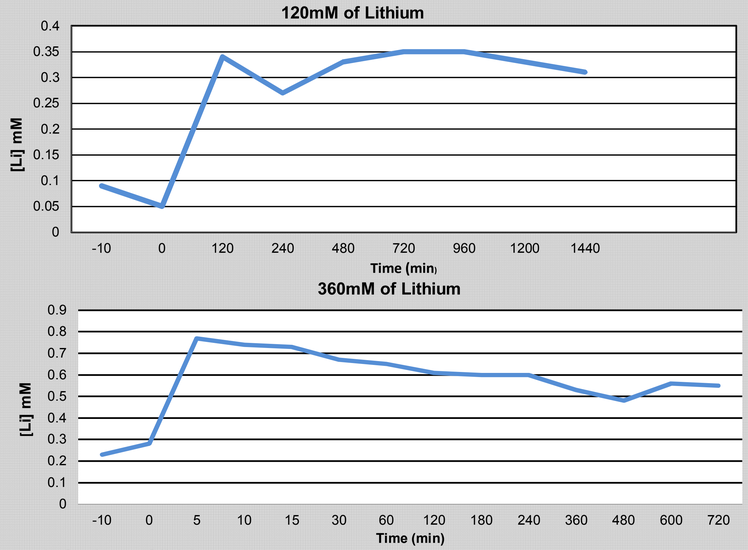
Phagotest®: This test kit allows the quantitative determination of leukocyte phagocytosis in heparinized whole blood and has been validated for horses in our laboratory. We have previous experience using this technique and find that it is a reliable method to determine phagocytic function in horses. This kit is will be used following the manufacturer's instructions. Briefly, heparinized whole blood will be incubated with FITC-labeled E. coli bacteria to stimulate phagocytosis by the white blood cells (negative control is incubated on ice); the phagocytosis is then stopped by placing the sample on ice and adding a quenching solution. This solution allows discrimination between attachment and internalization of bacteria by quenching FITC fluorescence (FL1) of surface bound bacteria (while not affecting the fluorescence of phagocytized bacteria). Washing is used to remove red blood cells, and a DNA staining solution (FL3) is added just prior to flow cytometry (see below).

Phagoburst®: This test kit allows the quantitative determination of leukocyte oxidative burst in heparinized whole blood and has been validated for horses in our laboratory. We have previous experience using this technique and find that it is a reliable method to determine oxidative burst function in horses. This test will be used following manufacturer's instructions. Briefly, heparinized whole blood will be incubated with various stimuli (E. coli bacteria, protein kinase C ligand phorbol 12-myrisate 13-acetate, N-formyl-MetLeuPhe) with a parallel sample incubated in the absence of stimuli as a negative control. Upon stimulation, polymorphonuclear granulocytes will produce reactive oxygen metabolites (superoxide anion, hydrogen peroxide, hypochlorous acid) that destroy bacteria inside the cell. Formation of reactive oxidants during oxidative burst will be monitored by the addition and oxidation of dihydrorhodamine (DHR) 123 as a fluorogenic substrate. The reaction is stopped by the addition of lysing solution, which also removes red blood cells. A DNA staining solution is then added. Oxidative burst capacity will be determined using flow cytometry (see below).

Methods Cont.

Flow cytometry: Flow cytometry will be performed at the University of Missouri Cell and Immunology Core Facility using a state-of-the-art flow cytometer CyAnTM ADP (Beckman Coulter) utilizing multiple laser excitation sources to analyze biological cells, beads, or other microscopic particles, and analyzed by SummitTM software. A minimum of 15,000 events will be recorded for each sample. The percentage of cells having produced reactive oxygen metabolites (recruitment) and the mean fluorescence intensity will be recorded. A marker for FL1 fluorescence should be set less than 1% of the events from control sample. This will determine what percentage of PMNs can perform phagocytosis, and how many bacteria each cell is able to ingest, in addition to evaluation of oxidative burst activity.

Preliminary Results



- Infusion of lithium did not cause any adverse 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
- Analysis of neutrophil phagocytosis and oxidative burst capacity is still in progress (results pending)

Acknowledgements and References

- Thank you to the Animal Health Foundation of St. Louis and the University of Missouri Veterinary Research Scholars Program for funding this study.

- Hatfield CL, McDonell WN, Lemke KA, Black WD (2001) Pharmacokinetics and toxic effects of lithium chloride after intravenous administration in conscious horses. Am J Vet Res 629: 1387–1392.
- 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