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

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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 immune 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 Patterns (PAMP) induced cytokine secretion in cultivated whole blood (Cwb) ex vivo. 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 6.8-12 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.

Methods

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.

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 (500 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.

References


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