Digital Expression of Isoforms of Cyclooxygenase in a Model of Bovine Laminitis

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Introduction

A great deal of the proposed pathophysiology of bovine laminitis is extrapolated from data obtained from equine laminitis. Similar to its equine counterpart, bovine laminitis commonly occurs secondary to conditions associated with endotoxemia (i.e. carbohydrate overload and mastitis). However, due to differences in clinical presentations of laminitis in the two species, our laboratory is interested to discern if the same pathophysiologic mechanisms take place in the two different species. Our laboratory has provided evidence that the developmental period of laminitis is an inflammatory event in horses. Specifically, we have demonstrated the upregulation of interleukin-1β (IL-1β) locally in equine laminae and systemically in mesenteric lymph nodes in the early stages of laminitis. Our lab also found increased COX-2 expression in equine laminae in horses in the developmental stage of black walnut extract-induced laminitis. Cyclooxygenase (COX) is the key enzyme in the process of converting arachidonic acid to eicosanoids. Downstream eicosanoid synthesis resulting from COX upregulation during laminitis may account for the aberrant vascular events occurring in the digit during this condition. The purpose of our project was to determine if cyclooxygenase isoforms (COX-1 and COX-2) were similarly affected in the laminitic bovid.

Materials and Methods

Thirty-one healthy steers were randomly divided into control and grain overload animals. Using a protocol that consistently results in clinical signs of laminitis, 21 steers were administered a grain mixture at 3.5% of their body weight. Control animals (n=10) were administered water only. Animals were anesthetized either after a decrease of at least 50% in the central venous pressure (6-hr laminitic group, n=12), at 12 hour post-administration of grain (12-hr laminitic group, n=9), or 6-8 hours after water administration (control group). Lamina and mesenteric lymph node tissue was collected from these groups. RNA was extracted and mRNA was isolated from the tissue samples. Real time quantitative PCR (LightCycler, Roche, Inc.) was used to assess COX-1, COX-2, and β-actin (housekeeping gene) expression in the lamina and lymph node tissues in the three groups of cattle.

Results and Conclusion

COX-2 expression in the laminae increased approximately 6-fold at six hours post-grain overload ($p=0.05$), and returned to pre-grain overload expression level by 12 hours post-overload (at the time of onset of clinical signs of laminitis). No significant differences occurred in COX-1 expression in the laminae between control and the grain overload groups. Although there was no significant difference in COX-2 expression in the mesenteric lymph nodes between the control and either of the grain overload time points, there was a significant decrease in COX-1 expression ($p < 0.05$) at both the six hour (approx. 6-fold decrease) and 12 hour time point (approx. 9-fold) in the grain overload groups when compared to the control value. The laminar upregulation of COX-2 in the developmental stage of bovine laminitis is similar to the results obtained in the development stage of black walnut extract-induced laminitis in the horse. The downregulation of COX-1 in the bovine lymph nodes subsequent to grain overload is surprising. However, similar to our results, endotoxemia has been reported to cause a marked decrease in COX-1 expression in multiple organs in rodent models.