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Doctor of Philosophy, Spring Commencement, 2009
Major: Veterinary Medical Sciences
Matrix Metalloproteinases in the Equine Systemic Inflammatory Response: Implications for Equine Laminitis
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Pages in dissertation, 124. Words in abstract, 316.

ABSTRACT

Laminitis is a crippling and often life-threatening disease of the equine foot. Soft tissue damage characteristic of this disease has been associated with increased MMP activity. Therefore, it seems likely that MMPIs could be potential therapeutic agents for laminitis. Further characterization of equine MMPs and evaluation of the effectiveness of MMPIs in the horse are needed.

Equine MMP-9 was harvested from neutrophils, purified by affinity chromatography, and evaluated using western blotting and gelatin zymography. The Biotrak MMP-9 Activity Assay was evaluated for use with equine samples using equine neutrophil MMP-9 as a standard, and was determined to have insufficient sensitivity for equine MMP-9. Therefore, zymography was used for evaluating MMP activity in all studies.

The abilities of doxycycline, oxytetracycline, and flunixin meglumine to inhibit LPS-induced equine MMP-2 and MMP-9 activities *in vitro* were investigated using a digital laminar explant model. The structural integrity of the explants was tested using an Instron biomechanical testing device, and MMP activity in the explants medium was evaluated using zymography. Doxycycline, oxytetracycline, and flunixin meglumine all successfully inhibited equine MMP-9 to varying degrees. However, only doxycycline and oxytetracycline increased the structural integrity of the explants. Explant structural integrity was inversely correlated with MMP-2 concentrations in the medium.

Based on the *in vitro* results, a non-terminal *in vivo* model for investigating MMPIs in the horse was validated. The administration of IV endotoxin to normal adult horses resulted in significant increases in MMP-2 and MMP-9 activities, as assessed by zymography. This *in vivo* model of MMP induction was used to determine the effects of doxycycline, oxytetracycline, flunixin meglumine, and pentoxifylline on equine MMP inhibition. Pentoxifylline and oxytetracycline appeared to be potent MMP-9 and modest MMP-2 inhibitors in the horse. Flunixin meglumine and doxycycline were potent inhibitors of equine MMP-2, but only weak inhibitors of equine MMP-9. These findings warrant the evaluation of pentoxifylline and oxytetracycline as MMPIs in the prevention/treatment of equine laminitis.