OXYTETRACYCLINE INHIBITS MMP-1 mRNA EXPRESSION AND COLLAGEN REMODELING BY EQUINE TENDON MYOFIBROBLASTS IN A DOSE-DEPENDENT MANNER: A MECHANISTIC BASIS FOR THE PHARMACOLOGIC TREATMENT OF EQUINE FLEXURAL DEFORMITIES

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Introduction Flexural deformities (contracted tendons) are a common crippling problem in foals. A flexural deformity represents a deviation of a limb in the sagittal plane and is expressed as a persistent hyperflexion of a joint region. While the etiology remains unclear, clinical and experimental studies have demonstrated that following large (3gm) intravenous doses of oxytetracycline (OTT) there is a "relaxation" of the metacarpal phalangeal angles in both affected (contracted) and unaffected foals [1]. It has been hypothesized that since OTT chelates calcium, relaxation of the joint angles occurs through the inhibition of calcium mediated muscle contraction [1]. However, since the key anatomic structure in controlling this angle, the deep check ligament (DCL), is not directly associated with a muscle and the extent of calcium chelation by OTT is minimal, this mechanism of action appears unlikely. Because neonatal foals respond to OTT more dramatically than older foals a more plausible theory involves the inhibition of collagen remodeling by myofibroblasts (MFb) which comprise the majority of cells within the DCL [2]. Tractional structuring of collagen is the mechanism by which cells exert tractional shearing forces on an extracelluar matrix resulting in the rearrangement and alignment of this matrix [3]. This is the proposed mechanism for the formation and maturation of ligaments and tendons and is dependent on MMP-1 expression [4]. Inhibition of collagen alignment in the rapidly growing neonate foal may make the DCL more susceptible to creep, thus explaining the relaxation of this structure in both normal and contracted foals following OTT administration. Since OTT has been shown to inhibit MMP expression in keratinocytes [5] it was our hypothesis that OTT inhibits MMP-1 expression in equine MFb, inhibiting the subsequent tractional structuring of collagen as measured by a standard gel contraction assay.

Materials and Methods Myofibroblasts were obtained from explant cultures of the DCL of 6 foals euthanized for reasons unrelated to this study. The cells were expanded to passage 3 and processed as follows. To determine the effect of OTT on the tractional structuring of collagen 1 ml collagen gels (2.4mg/ml type I bovine collagen) were seeded with MFb (200,000cells/ml), placed in individual 60mm culture dishes, and incubated with 0, 12.5, 25, or 75µg/ml OTT. These concentrations represent the maximum theoretical concentrations that would be seen by the cells and span the range of clinical dosages of OTT reported in the literature [1]. The 75µg/ml concentrations would reflect the clinical dose of 3 grams. After 24 hrs the gels were photographed and released from the bottom of the culture dishes. Additional photographs were taken at 1, 2, 4, 6, 8, and 24 hrs post-release (Figure 1). The digital images were used to measure the area of each gel over time. The effect of time and OTT dose on gel area was evaluated using an ANOVA. Significance was set at $p<0.05$. Five gels per dose per foal were examined and 3 replicates were performed on the cells from each foal.

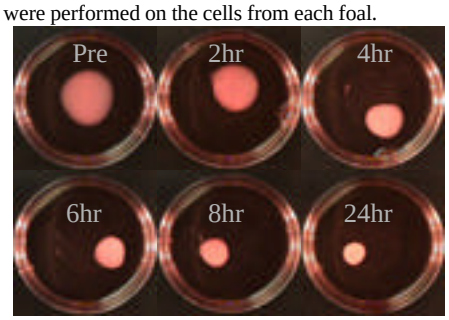

Figure 1 Photographs of a control gel (no OTT) showing the change in gel area due to tractional structuring over time.

To determine the effect of OTT on the expression of MMP-1, equine MFb (400,000cells/ml) were seeded into 1 ml gels and exposed to OTT as above. However, 2 hours after release the gels were harvested and total RNA was extracted and purified. RT-PCR was performed and MMP-1 expression normalized against GAPDH. The effect of OTT on MMP-1 mRNA expression was examined using linear regression with significance set at $p<0.05$.

Results OTT significantly inhibited gel contraction in a dose dependent fashion (Figure 2). While the extent of inhibition varied between foals with each OTT concentration, the concentration of 75µg/ml significantly inhibited gel contraction in all foals at all time periods. OTT significantly inhibited MMP-1 expression in a dose dependent manner ($R^2= 0.924$) (Figure 3).

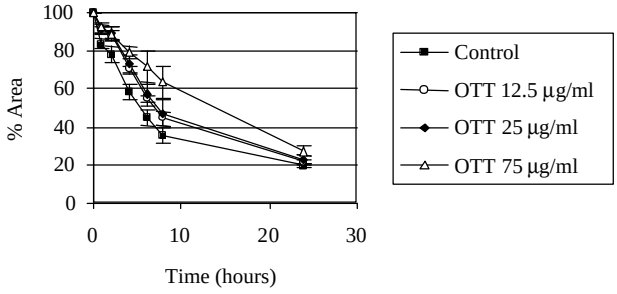

Figure 2 Representative graph from foal #2 showing the effect of various OTT concentrations on gel contraction.

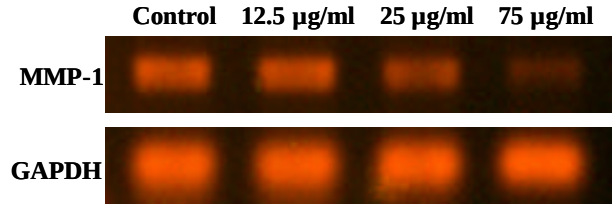

Figure 3 Representative RT-PCR gel of foal #5 demonstrating the dose dependent inhibition of MMP-1 mRNA expression by OTT.

Discussion The results of this study provide a mechanistic rationale for the clinical observations of joint angle relaxation in normal and contracted foals following large doses of OTT. The ability of OTT to inhibit tractional collagen structuring (as measured by gel contraction) appears to be directly related to the inhibition of MMP-1 mRNA expression by equine myofibroblasts. MMP-1 activity has been shown to be an integral component in a cell's ability to remodel and align the extracellular matrix of developing ligaments and tendons. Longitudinal growth of the limb bones of foals is most rapid during the first few weeks of life [6]. This is also the time when OTT therapy for flexural deformities has its most dramatic effect [1]. Therefore, any inhibition of MMP-1 expression during this time may impact the remodeling process and make the developing tissue more susceptible to creep. This increased susceptibility of the DCL to creep may explain the relaxation of this structure in both normal and contracted foals following OTT administration.

References

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