ORALLY ADMINISTERED SUB-ANTIMICROBIAL CONCENTRATIONS OF DOXYCYCLINE ATTAINS SYNOVIAL FLUID LEVELS CAPABLE OF INHIBITING MATRIX METALLOPROTEINASES 3 & 13.

*Schnabel LV; **Watts AE; **Papich M; *Torre CJ; *Mohammed HO; **Fortier LA
*Cornell University, Ithaca, NY
laf4@cornell.edu

Introduction
Osteoarthritis (OA) is a common cause of morbidity in humans and animals. Antibiotics belonging to the tetracycline family have been used to alleviate symptoms, and in some reports to slow the progression, of diseases such as OA, rheumatoid arthritis (RA), gingivitis, neurapathies, and acne. In vitro experiments have demonstrated that doxycycline has anti-matrix metalloproteinase (MMP) activities. Although the exact mechanism is not understood, the anti-MMP activity is believed to be the method by which doxycycline alleviates pain and slows the progression of cartilage degeneration in OA and RA. In vitro studies in our laboratory indicate that doxycycline at concentrations of 0.1µM (0.0462 µg/mL) significantly decreases MMP-3 and MMP-13 gene expression in synoviocytes treated with either interleukin-1 or MMP-13. Routine clinical application of doxycycline for treatment of OA has not been realized due to the concern in the medical community of antibiotic resistance. The use of sub-antimicrobial doses of doxycycline may obviate this concern; however, the pharmacokinetics of lower doses has not been studied. The purpose of this study was to determine if low-dose doxycycline administered orally could achieve sub-antimicrobial, anti-MMP concentrations in synovial fluid and plasma.

Methods
Six healthy adult horses without evidence of joint disease or lameness received doxycycline (5 mg/kg every 12 hours via nasogastric intubation; the antimicrobial dose in horses is 10 mg/kg every 12 hours). This dose was chosen based on pharmacokinetic knowledge of doxycycline in horses, and the lower limit of detection of doxycycline (0.1 µg/ml) using HPLC. Venous blood and synovial fluid samples were collected into heparin tubes immediately prior to doxycycline administration at t=0, and then at t=0.25, 0.5, 1, 12, 24, 48 and 72 hours. A different joint was used for synovial fluid aspiration at each time point to minimize blood contamination. Blood and synovial fluid samples were centrifuged (1000x g) and frozen at -80 C. Doxycycline concentrations were measured using HPLC in both plasma and synovial fluid. The sustained synovial fluid concentration was below 0.1490 µg/mL for all horses except horse 1 (0.2550 µg/mL (±0.0228)). At each time point following t=12 hours, the synovial fluid doxycycline concentration remained above 0.1227 µg/mL by t=0.5 hours, and the mean concentration at t=0.5 hours was 0.1943 µg/mL (±0.0279). At each time point following t=1 hour the synovial concentration remained above 0.1328 µg/mL except for horse 6. The mean concentration including all horses at the conclusion of the study (t=72 hours) was 0.2010 µg/mL (±0.0348) (Figure 2).

Results
Doxycycline concentration in the plasma of all horses was above 0.2146 µg/mL by t=0.5 hours, and the mean concentration at t=0.5 hours was 0.2550 µg/mL (±0.0228). At each time point following t=12 hours, the concentration was below 0.1490 µg/mL for all horses except horse 1 (Figure 1). Synovial fluid concentrations of doxycycline in all horses were above 0.1227 µg/mL by t=1 hour and the mean concentration at t=1 hour was 0.1943 µg/mL (±0.0279). At each time point following t=1 hour the synovial concentration remained above 0.1328 µg/mL except for horse 6. The mean concentration including all horses at the conclusion of the study (t=72 hours) was 0.2010 µg/mL (±0.0348) (Figure 2).

Conclusions
At the sub-antimicrobial dose (5 mg/kg) administered in this study, doxycycline concentrations were above the lower limit of detection for HPLC in both plasma and synovial fluid. The sustained synovial fluid concentration of greater than 0.1328 µg/mL in this experiment is greater than the concentration necessary to diminish MMP-3 and MMP-13 gene expression in synoviocytes treated with IL-1 or MMP-13 as previously determined in our laboratory. This study confirms that intra-gastric administration of low dose doxycycline results in measurable synovial fluid doxycycline concentrations which may decrease cartilage catabolism through inhibition of MMPs and slow the progression of OA. However, further in vivo studies are warranted to determine if MMP activity is inhibited in vivo and to fully elucidate a medication protocol.

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