VSL 130

Pharmacological Evaluation of Glyco-Flex® III on Canine Chondrocytes

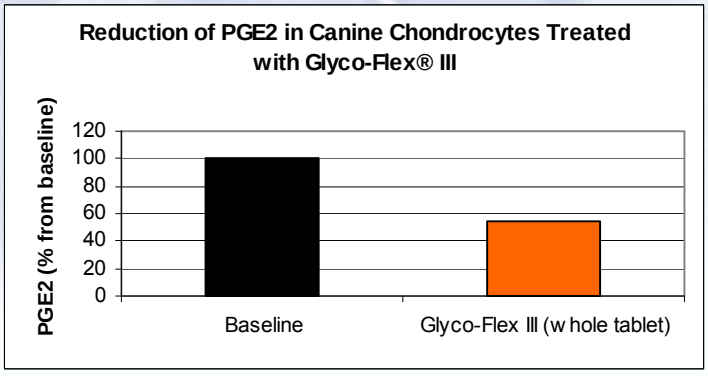
Objective: Canine chondrocytes were used in cell culture experiments to assess the effects of the Glyco-Flex® III tablets on key biological markers of inflammation.

Summary: This study was designed to assess the anti-inflammatory effects and antioxidant capacity of Glyco-Flex® III compared to deracoxib (Deramaxx®, Novartis Animal Health) as it relates to the production of key biological markers (including NO, SC, sGAG, cytokines, prostaglandins, and matrix metalloproteinase molecules) released after an inflammatory insult and produced in the pathogenesis of osteoarthritis on canine chondrocytes *in vitro*.

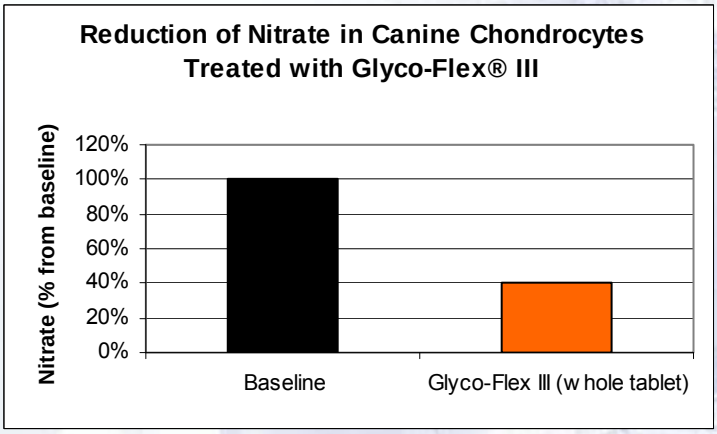
Background: Canine chondrocytes were used to simulate Osteoarthritis (OA) conditions *in vitro* in canine cartilage cells to test the effectiveness of Glyco-Flex® III in reducing biological markers associated with OA. OA is a progressive degenerative joint disease that afflicts more than 20% of the canine population older than 1 year. The use of joint support supplements has become very popular due to the associated gastrointestinal toxicity of some NSAIDs and other pharmacologic treatments. Glyco-Flex® III is a joint support supplement for dogs marketed by Vetri-Science® Laboratories of Vermont. Some of its active ingredients are widely recognized immune modulators, anti-inflammatory, and antioxidant agents.

Methods: Deracoxib whole tablet and Glyco-Flex® III whole tablet were dissolved in dimethylsulfoxide (DMSO) in concentrations 0-1000 μg/ml. Antioxidant capacity was measured using the ABTS method. Canine chondrocytes (CnC) were maintained in DMEM/F-12 medium (20% FBS, 10 mg/L penicillin-streptomycin). Inflammation in CnC (5,000 cells/well) was induced with Interleukin-1β (IL-1β) for 2 hours followed by compound treatment for 72 hours. The biological markers released into the cell culture medium were measured after 72 hours. Nitric oxide (NO) was measured as nitrate and nitrite levels utilizing Griess reagent, soluble collagen (SC) (Sircol) utilizing the Sirius red dye method, sulphated glycosoaminoglycans (sGAG) utilizing the Alcian blue dye method; and prostaglandin E_2 (PGE_2), interleukin-6 (IL-6), tumor necrosis factor-α (TNF-α), and matrix metalloproteinase-3 (MMP-3) were measured utilizing separate commercial ELISA kits.

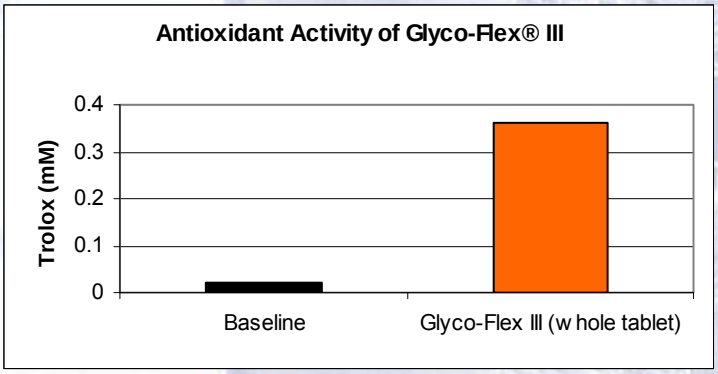
Results: In canine cartilage cells treated with Glyco-Flex® III there was a dose-dependant reduction in IL-6, PGE_2, MMP-3, TNF-α, and SC. Glyco-Flex® III maintained nitrate and sGAG at baseline levels. The antioxidant activity of Glyco-Flex® III whole tablet was found to be concentration-dependent.



Glyco-Flex® III whole tablet shows decreased levels of PGE_2 production as compared to baseline.



Glyco-Flex® III whole tablet shows decreased levels of Nitrate production as compared to baseline. Nitrate is a measurement of Nitric Oxide (NO).



Glyco-Flex® III tablets shows increasing antioxidant activity.

Conclusion: When used on a canine specific cartilage cell line, Glyco-Flex® III appears to have anti-inflammatory and antioxidant properties. Glyco-Flex® III tablets showed positive reductions in NO, SC, TNF-α, IL-6, PGE_2, and MMP-3, which are key markers of inflammation. In this canine specific model Gyco-Flex® III appears to reduce cartilage breakdown, inhibit cytokine induced NO and PGE_2 production, and reduce proteolytic breakdown after an inflammatory insult in similar potency as deracoxib. These *in vitro* results appear to demonstrate some of the key mechanisms by which Glyco-Flex® III functions in the joint.

Clinical Relevance: Results from this *In vitro* (VSL 130) study more than correlate/parallel other *in vitro* (VSL 260) & *in vivo* (VSL120) studies.

Yanez J, et al. Pharmacological Evaluation of Glyco-Flex® III on Canine Chondrocytes. Washington State University, 2006. Presented at NAVC 2007 and published in Journal of Medical Sciences, 2008: 1-14..