**VSL 260**

## Pharmacological evaluation of Glyco-Flex® III constituents on canine chondrocytes

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

**Summary:** This study was designed to assess the anti-inflammatory effects and the antioxidant capacity of the active ingredients of Glyco-Flex® III compared to deracoxib (Deramaxx®, Novartis Animal Health) as it relates to the production of key markers (including NO, SC, sGAGs, 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 inflammatory osteoarthritis (OA) conditions in canine cartiliage cells to test the effectiveness of the active ingredients of Glyco-Flex® III in reducing biological markers associated with OA. 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 the active ingredients in Glyco-Flex® III: *Perna cananiculus,* glucosamine HCl, methylsulfonylmethane (MSM), N,N-dimethylglycine (DMG), and grape seed extract (GSE) 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:** The active ingredients in Glyco-Flex® III maintained nitrate and sGAG at baseline levels. Perna, glucosamine, MSM, and DMG at all concentrations reduced nitrite and $PGE_2$ levels as high as 20% compared to baseline. There is a dose-dependant reduction in IL-6, MMP-3, TNF-α, and SC showing its potential anti-inflammatory activity. The antioxidant activity was found to be concentration-dependent, with GSE having the highest activity.

**Conclusion:** When used on a canine specific cartilage cell line, the active components of Glyco-Flex® III appear to have anti-inflammatory and antioxidant properties. Perna, Glucosamine, MSM, DMG, and GSE showed positive reductions in NO, SC, TNF-α, IL-6, $PGE_2$, and MMP-3, which are key markers of inflammation. The main active components of Gyco-Flex® III appear 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 may demonstrate some of the key mechanisms by which the main components of Glyco-Flex® III may function in the joint.

**Clinical Relevance:** The results from this *in vitro* (VSL 260) study more than correlate/parallel results from other *in vitro* (VSL 130) & *in vivo* (VSL 120) studies.

*Yanez J, et al. Pharmacological evaluation of Glyco-Flex® III constituents on canine chondrocytes. Washington State University, 2006. Presented at NAVC 2007and published in the Journal of Medical Science, 2008: 1-14.*