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 cartilage 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 glycosaminoglycans (sGAG) utilizing the Alcian blue dye method; and prostaglandin E2 (PGE2), 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 PGE2 levels as high as 20% compared to baseline. There is a dose-dependent 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, PGE2, and MMP-3, which are key markers of inflammation. The main active components of Glyco-Flex® III appear to reduce cartilage breakdown, inhibit cytokine induced NO and PGE2 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.