DMARD AUROTHIOMALATE INHIBITS COX-2, IL-6 AND MMP-3 EXPRESSION IN CHONDOCYTES BY INCREASING MKP-1 EXPRESSION AND DECREASING P38 PHOSPHORYLATION

R. Nieminen 1, R. Korhonen 1, T. Moilanen 2, A.R. Clark 3, E. Moilanen 1
1 Med. Sch., University of Tampere, Finland; 2 Coxa Hosp. for Joint Replacement, Tampere, Finland; 3 The Kennedy Inst. of Rheumatology Div., Imperial Coll., London, United Kingdom

Purpose: Aurothiomalate is a disease modifying antirheumatic drug that suppresses inflammation and retards cartilage degradation and bone erosion in arthritis. The molecular mechanisms of action of aurothiomalate are not known in detail. Mitogen-activated protein kinase (MAPK) pathways are major signaling pathways in inflammation, which regulate the production of many factors known to mediate inflammation and cartilage destruction in OA and RA. In the present study we investigated the effects of aurothiomalate on the activity of p38 MAPK and expression of MAPK phosphatase 1 (MKP-1), COX-2, MMP-3 and IL-6 in chondrocytes and intact cartilage.

Methods: Cartilage tissue was obtained from the leftover pieces of total knee replacement surgery from patients with OA and RA and from MKP-1-/- and wild type mice. Immortalized H4 murine chondrocytes* were used in cell culture experiments.

Results: Aurothiomalate inhibited IL-1β-induced COX-2 expression and prostaglandin E2 (PGE2) production by destabilizing COX-2 mRNA, as did the p38 MAPK inhibitor SB203580. Interestingly, aurothiomalate also increased the expression of MKP-1 and reduced IL-1β-induced phosphorylation of p38 MAPK. Knockdown of MKP-1 by siRNA significantly impaired the ability of aurothiomalate to inhibit the phosphorylation of p38 MAPK and the expression of COX-2, MMP-3 and IL-6. Likewise, aurothiomalate reduced COX-2, MMP-3 and IL-6 expression in human rheumatoid arthritis (RA) and osteoarthritis (OA) cartilage, and in articular cartilage from wild type mice but not in cartilage from MKP-1-/- mice.

Conclusions: The results provide a novel mechanism for the anti-inflammatory and anti-erosive action of aurothiomalate through increased MKP-1 expression, reduced p38 MAPK activation and suppressed expression of COX-2, MMP-3 and IL-6. MKP-1 may therefore be a promising novel target for the development of disease modifying drugs for RA and OA.

Eur J Pharmacol 2008; 584: 256-264

260

THE EFFECTS OF DEOXYNIVALENOL, NIVALENOL, T-2 TOXIN AND SELENIUM SUPPLEMENTATION ON IN VITRO TISSUE ENGINEERED CARTILAGE METABOLISM

J. Cao 1, M. Lu 1, F. Liu 1, Q. Fu 1, J. Liu 1, S. Li 1, J. Chen 1, A. Zhang 1, Z. Zhang 1, J. Zhu 1, C.E. Hughes 2, B. Caterson 2
1 Xi’an Jiaotong Univ., Xi’an, China; 2 Cardiff Univ., Cardiff, United Kingdom

Purpose: To investigate the effects of Nivalenol (NIV), Deoxynivalenol (DON) and T-2 toxin, in the presence and absence of Selenium (Se), on the metabolism of tissue engineered cartilage. These in vitro cartilage cultures mimic those found in Kashin-Beck Disease (KBD) environments that cause diarthrodial joint degeneration in skeletal joint development and osteoarthritis with ageing.

Methods: Bone matrix gelatin (BMG) was prepared from cancelloous bones of adult rabbits using previously published procedures (Li et al. J Zhejiang Univ Sci B. 2008, 9(1): 22-33). Human chondrocytes were isolated from a 10th-week human embryo cartilage anlage by sequential enzyme treatments. One million Passage 2 cells were seeded onto BMG grafts for pre-culture for 48 hours. Three toxins (DON, NIV & T-2 toxin), in the presence or absence of Se, were added: DON, 1.0 μg/ml; NIV, 0.1 μg/ml; T-2, 0.01 μg/ml; Se, 0.1 μg/ml. After 3 weeks culture in vitro, the BMG with the tissue-engineered cartilage graft was fixed, embedded, and cut into 14 μm slices for histological analysis. Slides were stained with hematoxylin & eosin (H&E) and toluidine blue. Immunohistochemical analysis was used to detect the expression of types II & X collagen, aggrecan, MMP1, MMP3, TIMP1, TIMP3 and α2 macroglobulin.

Results: H&E staining showed that the tissue-engineered cartilage grafts were very similar to normal cartilage in vivo, with chondrocytes localised in lacunae, separated from each other by the interterritorial extracellular matrix. There were no necrotic chondrocytes observed in the Control or the Toxin/Se treated groups. Control tissue engineered cartilage grafts expressed aggrecan and type II collagen, and a small amount of type X collagen. Immunohistochemical analysis of grafts exposed to DON, NIV and T-2 toxin showed decreased expression of type II collagen and aggrecan. However, Se addition restored type II collagen and aggrecan staining in the presence of these Toxins. There was increased MMP-1 and MMP-3 expression in the Toxin-treated grafts.