DISEASE MODIFYING EFFECTS OF A SELECTIVE COX-2 INHIBITOR IN RAT ANTERIOR CRUCIATE LIGAMENT transection (ACLT) MODEL OF OSTEOARTHRITIS.


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Introduction Osteoarthritis (OA) is a degenerative joint disease characterized by articular cartilage degradation, subchondral bone sclerosis, and osteophyte formation. It is widely accepted that cartilage destruction in OA is caused by an imbalance between the anabolic and catabolic pathways. Prostaglandin E2 (PGE2), produced by activated chondrocytes and synoviocytes, likely induces cartilage metabolism, synovial inflammation, and subchondral osteoclastic bone resorption. Thus PGE2 together with nitric oxide and proinflammatory cytokines such as IL-1 may exert profound effects on the pathophysiology of joint erosion in OA. Moreover, expression of cyclooxygenase-2 (COX-2), the predominant mediator of PGE2 production, is known to increase in OA joints. To investigate the potential contribution of inflammation and bone turnover to OA progression, we examined the effects of MF tricyclic, a potent and selective inhibitor of COX-2, in combination with Alendronate (ALN), on cartilage degradation and periarticular bone changes in the rat surgically induced OA model.

Materials and Methods All procedures were approved by the Institutional Animal Care and Use Committee of Merck Research Laboratories. Thirty-five 20-week old male Sprague-Dawley rats were subjected to either anterior cruciate ligament transection (ACLT) or sham-operation in the right knee. Rats were divided into 5 groups. Sham treated with vehicle (Sham+V), ACLT treated with vehicle (ACLT+V), ACLT treated with MF tricyclic at 3 mg/kg, daily p.o. (ACLT+MF), ACLT treated with ALN at 0.12 mg/kg/wk, twice per week, s.c. (ACLT+ALN), ACLT receiving both ALN and MF tricyclic using the indicated doses respectively (ACLT+ALN+MF). Each group had 7 rats and were sacrificed 10-wk post-surgery. After disarticulation, femora were evaluated for incidence of osteophyte formation by gross morphology. Tibiae were cut in half at the center of articular surface along with the medial collateral ligament in frontal section with a band saw. Anterior parts were embedded in paraffin for staining with toluidine blue-O and immunohistochemistry and posterior parts were embedded in methylmethacrylate for Masson’s trichrome staining for further bone histomorphometry. Semi-quantitative histopathological grading (modified Mankin criteria) was scored from 3 sections 100 µm apart. Subchondral bone changes were evaluated by image analysis using Image Pro plus as previously described (Ref). Osteophyte surface area was measured in Masson’s trichrome stained sections by manual tracing. We also determined the levels of urinary C-terminal telopeptides derived from collagen type I (CTX-I) and type II (CTX-II), known biomarkers of degradation of bone and cartilage, respectively. Using immunohistochemical methods, localization of COX-2 and the membraneous prostaglandin E synthase (mPGES) were examined in OA and normal articular cartilage derived from ACLT- and sham-operated rats, and from human OA and normal cartilage tissues. Furthermore, the potential direct effect of ALN (30 µM) on expression of COX-2 and PGES, and the levels of PGE2 in human primary chondrocytes were examined using real time Taqman PCR and PGE2 immunoassay, respectively.

Results Initially, we found that COX-2 expression was up-regulated in the articular cartilage of ACLT- as compared to sham-joints using real time quantitative PCR (2-fold). Using immunohistochemistry, we also detected the increase in COX-2 protein in articular cartilage derived from both rat and human OA joint tissues. Similar to ALN, MF tricyclic had significant chondroprotective effects on the ACLT-joints. Both the COX-2 inhibitor and ALN partially reduced the histological Mankin score (p<0.05 and 0.001, respectively) of cartilage damage during OA progression, and suppressed the elevated levels of urinary CTX-II, a cartilage degradation marker, 2-wk post-surgery. While MF tricyclic did not inhibit generalized bone resorption, as determined by urinary CTX-I, this compound (p<0.0001) significantly inhibited local subchondral bone sclerosis in the ACLT-joints as evaluated by histomorphometry, by comparison to ALN (p<0.001). Osteophyte formation was also suppressed by both COX-2 inhibitor and ALN. Interestingly, there were no additive effects observed in chondroprotection and osteophyte formation in ACLT-joints treated with the combination of both drugs. Immunohistochemical analysis revealed that protein levels of COX-2 and mPGES in articular chondrocytes were inhibited in the ALN-treated ACLT-joints. Moreover, we observed that ALN reduced COX-2 mRNA levels and PGE2 production in IL-1-treated primary culture of human articular chondrocytes.

Discussion This study suggests that COX-2-mediated pathway is up-regulated in both human and rat OA tissues, and inhibition of this enzyme results in structure-modifying effects in the rat OA model. In addition, the lack of additivity of the two compounds in vivo, together with the inhibition of COX-2 activity by ALN in vitro, suggest that ALN may directly modulate the COX-2/PGE2 pathway in mediating cartilage/bone metabolism in the rat ACLT model.

References Hayami T. et. al. Arthritis and Rheumatism 2004; 50, p.1193