tis (OA), cartilage injuries, osteochondral defects, intra-articular fractures and intra-articular drill holes may predispose a joint to hyaline cartilage damage.
Repeated bleeding into joints in hemophiliacs causes chronic synovitis, leading to joint destruction. Bone bleeding may cause early chondral damage. This suggests that changes in the environment of the joint drive articular cartilage degeneration.
Our purpose was to create and characterize a controlled intra-articular bone injury to define effects on cartilage damage, to determine the impact of bone trauma in the knee without altering joint loading and mechanics. We hypothesize synovitis caused by the injury will lead to cartilage degeneration as seen in early OA. We also aimed to prevent early leukocyte recruitment and activation with a novel polypeptide treatment (feG). We hypothesize that downregulation of leukocytes in the joint will reduce post-surgical joint inflammation and prevent cartilage degradation.
Methods: New Zealand White rabbits underwent surgery, coring two 1.1mm diameter, 10mm deep holes 7mm apart into the right femoral notch. Shams and non-operated controls were run concurrently. Blood samples were analyzed weekly. Rabbits were euthanized 3, 6, 9 wks, and 1 yr post-surgery (p-s) (n=3/group) and knee joints were assessed for osteophytes and gross signs of cartilage degeneration; cartilage and synovium were collected. Histological sections of cartilage were graded by Mankin score. Molecular analysis was performed on cartilage samples. Histological sections of synovium were graded for inflammation and molecular samples were analyzed for inflammatory markers by ELISA. Blood was collected weekly for leukocyte analysis. Apoptosis was characterized in the knee joints with an IR dye (Flivo™) in a small group of rabbits 24 hrs, 2 wks, and 3 wks p-s.
A second group of rabbits was treated with feG or saline (controls) just prior to surgery and 12 hrs p-s. Animals were euthanized 72 hours p-s and synovial cells and blood cells were analyzed by FACS. Synovium, cartilage and fat pad were collected for histological and molecular analysis.
Results: 6 weeks p-s experimental animals had cartilage thinning in the experimental knee. By 9 weeks p-s the control joints of the experimental animals had osteophytes on the outer sides of the femoral condyles and tibial plateaus, cartilage discoloration, and marked cartilage thinning. Fibrocartilage formed on the bone core sites. By 1 year p-s the joint showed osteoarthritis with large amounts of fatty infiltration, fibrosis, osteophytes, and cartilage damage. These data were reflected in histological cartilage data which show cartilages were significantly different ($p = 0.014$) with scores significantly higher after 3, 6 and 9 weeks p-s. Whole white blood cell counts indicate that animals have a systemic inflammatory response immediately after surgery. This inflammatory response was not seen in the synovium at the time of euthanasia; however the synovium appears to have become fibrotic.
Gross differences were not apparent after treatment with feG; however, we expect to see differences with more sensitive measures.
Conclusions: These findings suggest that changing the environment of the knee joint by non-chemical, non-destabilizing surgical injury can cause early cartilage degeneration which has the potential to develop into OA. Also that downregulating early leukocyte recruitment and activation may help reduce post-surgical inflammation leading to OA.
This novel femoral notch coring surgery provides a relevant clinical model that we have characterized and used for modulating post-surgical inflammation leading to later cartilage degeneration.

103

DESIRABLE EFFECT OF COMBINATION THERAPY WITH HIGH MOLECULAR WEIGHT HYALURONATE AND NSAIDS ON MATRIX METALLOPROTEINASES PRODUCTION

M. Mihara, M. Hashizume, N. Koike, H. Yoshida, M. Suzuki
Chugai Pharmaceutical Co., Ltd, Gotemba, Japan

Purpose: We examined the combination effects of intra-articular high molecular weight hyaluronate (HA) and oral NSAIDs on cartilage destruction and pain of knee joint in rabbit knee osteoarthritis (OA) model induced by partial meniscectomy. We further studied the effect of HA and NSAID on MMPs production by IL-1 and IL-6 from human chondrocytes in vitro.
Methods: Rabbit OA model was induced by partial meniscectomy and treated with oral NSAIDs, intra-articular HA and the combination of NSAID and HA over 2 weeks. NSAID was orally administered daily from the day of meniscectomy for 14 days and HA was intra-articularly injected 5 times on day 0, 3, 6, 9, 12. Pain of knee joint was assessed by an Incapacitance Tester and the damage of cartilage was evaluated by visual assessment and histopathology at 14 days after the surgery. Cartilage destruction and the levels of MMP-1, MMP-3 and MMP-13 in synovial fluid were measured. In *in vitro* study, human articular chondrocytes were cultured with NSAID and/or HA in the presence of IL-1β or IL-6+soluble IL-6 receptor (sIL-6R) for 24 h. After culture, production of MMPs, IL-1β, and IL-6 in the supernatants was measured.
Results: Hind paw weight bearing on the injured paw decreased time-dependently in the control group. In HA, NSAID and HA+NSAID group, the decrease of hind paw weight distribution was suppressed, demonstrating an analgesic effect. Visible and histopathological damages of cartilage were evident in the control group. In the HA group, the cartilage destruction was ameliorated. In contrast, in the NSAID group, the cartilage destruction was augmented compared with the control group. And this exacerbated cartilage destruction was reversed by the concomitant use of HA. Consistent with histopathology, the levels of MMP-1, MMP-3 and MMP-13 in synovial fluid from the NSAID group were significantly higher than those from controls. In contrast, the HA and HA+NSAID groups had lower levels of all three MMPs than the NSAID group. To examine the direct effect of NSAID on MMP production in chondrocytes, celecoxib and indomethacin were added to human chondrocytes in the presence of IL-1β or IL-6+sIL-6R. celecoxib and indomethacin significantly enhanced all three MMPproductions induced by IL-1β or IL-6+sIL-6R. PGE_2 production by IL-1β and IL-6 + sIL-6R was inhibited by all drugs. We also examined whether the addition of PGE_2 reduced MMP production induced by NSAID. However, PGE_2 did not affect the production of MMPs augmented by NSAID. When we measured IL-1β and IL-6 production in supernatants,NSAID potently augmented IL-1β-induced IL-1β and IL-6-induced IL-6 production. Furthermore,we examined the influence of HA on cytokine-induced MMPs production by chondrocytes. MMP production by IL-6+sIL-6R was inhibited by either HA or anti-IL-6R antibody. Similarly, MMP production by IL-1β was inhibited by either HA or IL-1R antagonist.Next, we examined the effect of HA on NSAID-accelerated MMP production. Cytokine-induced MMP production was augmented by NSAIDs and NSAID-augmented MMP production was significantly suppressed by the addition of HA. HA also significantly suppressed the NSAID-augmented production of IL-1β and IL-6. This increased production of MMPs induced by NSAID was counteracted by the combination of HA.
Conclusions: These results indicate that HA has an advantage to prevent NSAID-augmented cartilage destruction via the inhibition of MMP production and the combination therapy of HA and NSAID is strongly recommended.

104

COL2-PD2EGFP REPORTER MICE: SUITABLE MODEL FOR STUDIES IN CHONDROCYTE GROWTH PLATE BIOLOGY

M. Tryfonidou[1], G.P. Lunstrum[2], K.H. Hendriks[1], F.M. Riemers[1], C.R. Degnin[2], L.C. Penning[1], H.A. Hazewinkel[1], W.A. Horton[2]
[1]Utrecht Univ., Faculty of Vet. Med., Utrecht, Netherlands; [2]Oregon Hlth.& Sci. Univ., Dept of Molecular & Med. Genetics, Portland, OR

Purpose: Can Col2-pd2EGFP mice be employed for studying articular and growth plate chondrocyte biology?
Methods: Transgenic Col2-pd2EGFP mice have pd2EGFP ($t_{1/2}$~2 hrs) downstream of the Collagen 2 promotor. The fluorescence pattern of these mice was studied during postnatal development with confocal microsopy of the knee joints and femoral head. Pd2-EGFP chondrocytes were FACS-sorted and cultured on glass chamber slides in high-density microcultures of 20.000 cells each. Directly after FACS (T-16h) and 16 hrs after initiating culture at T0, 2, 7, 14, and 21 days q-RT-PCR *gfp*, *col2α1*, *colX*, and *col1* expression and laser confocal scanning was performed for Col 2 and Col 10 matrix deposition.
Results: At 1 until 4 weeks of age a dramatical drop in GFP expression of the articular cartilage occurs reflecting the decrease in Col2α1 production by articular chondrocytes during growth. Confocal microscopy of the growth plates reveals GFP-expression is in all proliferative (Pr) and some hypertrophic chondrocytes (Hyp).
In vitro, *gfp* levels do not quantitatively correlate with those of *col2α1* probably due to the fact than one transcripts of GFP have been incorporated in the genome. *gfp* increases by 20-fold at T0 compared to T-16h, decreases gradually until T7 and becomes almost undetectable thereafter. The recovered pd2EGFP-growth plate chondrocytes were cultured successfully at least for 21 days in microcultures. Pd2EGFP-chondrocytes start producing Col2 by T2 as illustrated here.