in H&E stained sections. Area of CCD was divided by total articular cartilage area to generate a percentage. In addition, a larger group of joints (n=158), including all joints from the present study, was evaluated using a comprehensive histological grading scheme composed of both semi quantitative grades and continuous measurements (Study 2). The data from the medial tibial plateau was then subjected to Principal Components Analysis with one of the resulting factors being weighted by the following variables: total chondrocyte area, percentage of area of chondrocyte cell death, total number of viable chondrocytes, total articular cartilage area per viable chondrocyte, viable articular cartilage area per viable chondrocyte. From the factor analysis, individual factor scores for the joints evaluated in Study 2 were generated. The mean percent CCD areas and the individual factor scores from the factor weighted by variables relevant to CCD were evaluated by ANOVA with age as the independent variable.

Results: The results from Study 1 revealed significant differences in percentage of CCD within the articular cartilage in the medial tibial plateau vs. age, with similar trends in the lateral tibial plateau. The youngest mice had the smallest area of CCD and this progressively increased with age (Fig. 1). All comparisons were significant except for those involving the 16 month old mice, which were not significantly different from any of the other age groups. The data using the factor score for CCD were closely similar (Fig. 2).

Conclusions: Increased area of chondrocyte cell death with age in murine articular cartilage has not previously been reported and is an important consideration in studies of aging and osteoarthritis in this species.

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DEXAMETHASONE INHIBITS AGGREGAN DEGRADATION IN HUMAN ARTICULAR CARTILAGE BUT NOT IN ANIMAL TISSUE

Y. Sawaji1,2, R. Faisal2, J. Hynes2, J. Saklatvala2
1Tokyo Univ. of Med., Tokyo, Japan; 2Kennedy Inst. of Rheumatology, London, United Kingdom

Purpose: Glucocorticoids are widely used for treatment of rheumatoid arthritis to suppress inflammation. Little is known about their action on cartilage metabolism and the therapeutic efficacy for treatment of osteoarthritis (OA), although they have adverse effects on other connective tissues, such as skin and bone. The effects of the synthetic glucocorticoid dexamethasone (Dex) on cartilage degradation were investigated by examining aggrecan catabolism and expression of aggrecanases (a disintegrin and metalloproteinase with thrombospondin motifs (ADAMTS)-4 and -5) and collagenase (matrix metalloproteinase (MMP)-13) in human, porcine and mouse articular cartilage.

Methods: Rested articular cartilage explants from human, porcine or mouse joints were stimulated with either interleukin (IL)-1 or retinoic acid (RetA) in the presence or absence of Dex (1, 10, 100 nM) with or without RU-486 (a glucocorticoid receptor antagonist). Aggrecan degradation was quantified by measuring the release of glycosaminoglycan (GAG) and aggrecanase-dependent aggrecan fragments by Western blotting with neo-epitopes antibodies for AR/LGS and AGEG. The expression of ADAMTS-4,-5, MMP-13 and cyclooxygenase (COX)-2 mRNAs was measured by quantitative real-time PCR.

Results: IL-1 caused aggrecan degradation with the release of GAG and aggrecanase-dependent aggrecan fragments (AP/LGS and AGEG) and induction of ADAMTS-4,-5, MMP-13 and COX-2 mRNA in all species tested. The induction of aggrecanase-dependent aggrecan degradation was suppressed by Dex in human, accelerated in porcine and unaltered in mouse cartilage explants. Dex inhibited the induction of ADAMTS-5 but not ADAMTS-4 mRNA in human, augmented both ADAMTS-4 and ADAMTS-5 mRNA in porcine and inhibited ADAMTS-4 but not ADAMTS-5 mRNA in mouse cartilage. Dex inhibited the IL-1-induction of MMP-13 and COX-2 mRNA in all species as expected. RetA caused aggrecan degradation and induction of ADAMTS-5 mRNA in all species and they were also inhibited by Dex in human, enhanced in porcine and unaffected in mouse cartilage. Actions of Dex were reversed by pretreating cartilage with RU-486 in all species.

Conclusions: Our findings suggest that glucocorticoids may be therapeutically beneficial by inhibiting aggrecan degradation and induction of aggrecanase mRNA and protein expression in human articular cartilage. In marked contrast, Dex did not inhibit aggrecanolysis in mouse cartilage and further accelerated it in porcine tissue. These species differences need to be carefully considered when working on the regulation of aggrecan catabolism by aggrecanases.

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CHANGES IN TGF-BETA SUPERFAMILY SIGNALING BALANCE FROM SMAD2/3 TO SMAD1/5/8 ARE ASSOCIATED WITH CHONDROCYTE MMP-13 EXPRESSION

E.N. Blaney Davidson, E.L. Vitters, H.M. van Beuningen, R. Janssen, W.B. van den Berg, P.M. van der Kraan
Radboud Univ. Nijmegen Med. Ctr., Nijmegen, Netherlands

Purpose: Osteoarthritis (OA) is characterized by cartilage degradation. Changes in TGF-beta superfamily signaling regulate chondrocyte differentiation and play a role in OA development. The major TGF-beta signaling route is via Smads, Smad1/5/8, and Smad2/3 the TGF-beta route. We have shown that Smad2/3 signaling was reduced in OA. In contrast, BMP2, known to stimulate Smad1/5/8 signaling, was elevated nearby OA lesions. We studied whether BMP-2 expression is related to diminished Smad2/3 signaling and explored the potential biological significance of BMP-2 neighboring OA lesions.

Methods: C57Bl/6 mice were injected with Ad-BMP-2 i.a. Knee joints were isolated for histology 3 and 7 days after injection and stained for Smad2/3 and aggrecan degradation neoeptopes VDIPEK and NITEGE. The impact of elevated BMP2 was investigated in chondrocytes in culture, stimulated with Ad-BMP-2. RNA was isolated for Q-PCR analysis. As BMP2 reduced Smad2/3, we investigated the effect of reduced Smad2/3 signaling on Smad1/5/8 signaling by exposing chondrocytes to SB-505124, an ALK5 inhibitor, followed by Western blot analysis of Smad2/3P and Smad1/5/8P.

To elucidate whether BMP2 modulates effects of TGF-beta/Smad2/3 signaling in cartilage, we injected C57Bl/6 murine...