to obtain more than two echoes in a fast-spin-echo sequence. With these limitations in mind, two four-echo sequences were run with echo times of 16, 32, 48, 64ms and 20, 40, 60, 80ms. In both cases, TR=2000ms, FOV=38cm, thickness=3.5mm and matrix=256x256. These series were collected with fat saturation turned on and again with fat saturation turned off. In addition, the series were collected as a single slice and as a multi-slice acquisition. In addition to the four-echo spin-echo sequences, three dual-echo fast-spin-echo sequences were obtained. All FSE series used TR=4500ms, FOV=38cm, thickness=3.5mm, ETL=8, matrix=256x256, and fat saturation turned on. The three series had echo times of 16 and 82ms, 24 and 59ms, and 16 and 99ms.

In addition to the phantom study, a bovine study was carried out using the same imaging parameters on a bovine knee.

Results: For the phantom study, the ground truth results were compared with the T2 decay times calculated from each of the series. A regression was performed for all vials that had a ground truth T2 value of less than 200ms. When estimating the T2 values using all four of the four-echo spin-echo sequence with echo times of 16,32,48,64ms and 64ms, the slope is 0.5628 and R-squared is 0.9808. It has been shown that dropping the first echo when estimating T2 provides significantly better results. By using only the echoes at 32, 48, and 64ms, the slope is 0.8819 and R-squared is 0.9702. When using the 40, 60, and 80ms echoes, the slope is 0.8677 and R-squared is 0.9759. These two results do not differ substantially, although the slope is slightly closer to one for the shorter echo times.

This was compared to the single-slice data for the 32, 48, and 64ms echoes. The slope was 0.4496, the intercept was 18.2939ms and R-squared was 0.9835. For the dual-echo sequences, the best results were obtained from the echoes at 16 and 82ms. The slope was 0.9015, the intercept was 5.8687ms and R-squared was 0.9971.

Results will be obtained for the bovine knee for all of the cartilaginous regions. A direct comparison of all the techniques will be obtained at the pixel level.

Conclusions: From the results of the phantom study, the dual-echo fast-spin-echo sequence with echo times of 16 and 82ms provided the most accurate and consistent results for the vials with echo times below 200ms. Large magnetization transfer contrast effects were observed in the 4-echo spin-echo sequences by comparing the single slice acquisitions to the multi-slice acquisitions. The results were substantially worse for the single-slice acquisitions. This same phenomena was not observed in the dual-echo sequence. Our hypothesis is that the magnetization transfer contrast counteracts the stimulated and additional spin echoes that occur in the 4-echo multi-slice sequences.

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HEME OXYGENASE-1 DOWN-REGULATES MICROSMAL PROSTAGLANDIN E SYNTHASE-1 IN OSTEOARTHRITIC CHONDROCYTES

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Purpose: Pro-inflammatory cytokines are believed to play a role in the pathogenesis of osteoarthritis (OA). We have previously shown that these cytokines down-regulate heme oxygenase-1 (HO-1) in OA cartilage and chondrocytes. We have also shown a protective effect of HO-1 induction on cartilage degradation. Pro-inflammatory cytokines induce the coordinated expression of cyclooxygenase-2 (COX-2) and microsomal prostaglandin E synthase-1 (mPGES-1) leading to PGE2 overproduction. Recent data indicate that the predominant effects of this prostanoid in OA chondrocytes are catabolic. We have examined the effects of HO-1 on the production of oxidative stress and PGE2 in OA chondrocytes and the mechanisms involved.

Methods: Chondrocytes were isolated by digestion with collagenase and used in primary culture. Cells were stimulated with IL-1α. HO-1 was induced by incubation with 10 μM cobalt protoporphyrin IX (CoPP). PGE2 was measured by RIA. Gene expression was analyzed by real-time PCR. Protein expression was investigated by Western blot, ELISA and immunocytochemistry. Apoptosis and oxidative stress were determined by LSC. The activation of nuclear factor-κB (NF-κB), activating protein-1 (AP-1) and early growth response (EGR) was assayed by the luciferase method. To determine the effects of HO-1 overexpression, three-dimension cultures of chondrocytes were transduced with a lentiviral vector (LV-HO-1).

Results: Induction of HO-1 by CoPP augmented viability and aggreccan content, and decreased the production of oxidative stress and PGE2. This effect was not dependent on the inhibition of COX-2/mPGES-1 enzyme activity. HO-1 induction did not modify COX-2 expression but it significantly decreased mPGES-1 at the protein and mRNA levels. Induction of HO-1 resulted in a significant reduction of EGR-1-luc and NF-κB-luc promoter activation. In cells transduced with LV-HO-1, mPGES-1 expression and PGE2 production were significantly inhibited.

Conclusions: HO-1 decreases the production of inflammatory mediators in primary OA chondrocytes stimulated with IL-1α. The inhibition of PGE2 production would be dependent on mPGES-1 down-regulation. The inhibition of EGR-1 activation provides a basis for this effect of HO-1.

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THYMOQUINONE INHIBITS INFLAMMATORY AND CATABOLIC RESPONSES AND LIPID PEROXIDATION IN RHEUMATOID ARTHRITIS

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Purpose: Tymoquinone (TQ) is the major active compound derived from the medicinal Nigella sativa. Few studies have been shown that TQ exhibits anti-inflammatory activities in experimental model of rheumatoid arthritis (RA) through mechanisms that are not fully understood. The aim of this study was to evaluate the in vitro and in vivo effects of TQ and to investigate its influence on the major signaling pathways involved in RA pathophysiologic changes.

Methods: Isolated human RA synoviocytes were pre-incubated with increasing concentration of TQ (0-20 μM) and then incubated with 1 μM lipopolysaccharide (LPS) for 24 h. Experimental model of RA was induced by 0.5 mg native chick collagen II (CII) solubilized in 0.1 M acetic acid and emulsified in incomplete Freund’s adjuvant and scores of arthritis were recorded. RA rats were randomly distributed into 2 groups and treated orally with 1) water + 0.1% ethanol, or 2) 5 mg/kg/day of TQ. Pro-inflammatory cytokines, matrix metalloproteinase-1 (MMP-1), prostaglandin E2 (PGE2) were measured in culture media and serum by commercial kits. Cyclooxygenase (COX-2) and phosphorylated levels of mitogen activated protein kinases (MAPKs) and nuclear factor-kappaB (NF-κB) were assessed by Western blot. mRNA levels of pro-inflammatory cytokines, MMP-1, COX-2 were assessed by real-time reverse transcriptase-polymerase chain reaction (RT-PCR). To investigate bone metabolism and resorption, serum pro-inflammatory cytokines, alkaline phosphate (ALPase),...
receptor activator of nuclear factor-kappaB ligand (RANKL) were determined with kits. Lipid peroxidation product 4-hydroxynonenal (HNE) was measured in cellular extract and serum using an in house ELISA.

Results: In isolated RA synoviocytes, TQ addition showed a dose-dependent decrease in LPS-induced interleukin-1beta (IL-1β), IL-6, tumor necrosis factor alpha (TNFα), MMP-1, COX-2 expression and PGE2 release. The phosphorylated levels of p38, p42/44, p46/54 MAPK, as well as p65 were also decreased by TQ addition. In our experimental model of RA, the oral administration of 5 mg/kg/day of TQ slightly attenuated paw swelling and significantly reduced the serum levels of IL-1β, TNFα, APLase and RANKL. Finally, the generation of HNE was dramatically prevented by TQ administration in RA synoviocytes and serum.

Conclusions: This study reports the anti-inflammatory, anti-catabolic and antioxidant effects of TQ and suggests that it may be a clinically valuable agent in the prevention of articular diseases including RA and osteoarthritis.

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DEVELOPMENT OF A HISTOLOGICAL GRADING SCHEME FOR OSTEOARTHRITIS IN MICE

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Purpose: Histological grading schemes that are used for osteoarthritis (OA) need to be improved in order to accurately characterize the severity of this disease. The purpose of this study was to develop a histological grading scheme to accurately assess the severity of OA in mice.

Methods: This study utilized murine stifle joints from 5 studies (n=159 stifle joints) that included both surgically induced (destabilization of the medial meniscus) OA in 10-12 week old mice and naturally occurring OA in older adult mice and represented a wide range of OA severities. Stifle joints were routinely fixed, decalcified, embedded, sectioned in a coronal plane. Two representative midcoronal sections, as determined by bony and soft tissue landmarks, were selected for evaluation and stained with hematoxylin & eosin (H&E) and Safranin-O stains and all slides were randomized. Fifteen parameters composed of both quantitative and semi-quantitative measurements were evaluated in each medial and lateral tibial plateau of each stifle. Results from all parameters for both plateaus and all stifles were combined into one data set and evaluated using correlation analysis and Principal Components Analysis (PCA) by a statistician having no knowledge of the intervention groups.

Results: Correlation analysis revealed strong correlations in the medial tibial plateau (p<0.0001) between the two semi-quantitative parameters (Articular Cartilage Structure and Safranin-O staining scores) and 6 continuous parameters. Correlations among these parameters were weaker in the lateral tibial plateau. Five factors were retained by PCA using data from the medial tibial plateaus only, accounting for 74% of the total variance in the data (Table 1; loading score for each parameter in parentheses, percent variation accounted for by each factor located in the bottom row). These grouped logically into factors describing articular cartilage structure, cell viability, subchondral bone, meniscus, and osteophytes.

Conclusions: A comprehensive histological grading scheme was developed to describe joint changes of OA in mice. Analyses focused on the medial joint compartment since lesions were more severe in this site than in the lateral joint compartment. PCA allowed the generation of factors describing OA severity in the medial tibial plateau that had conceptual meaning and also provided information regarding which combinations of features accounted for greater and lesser variation in the data. The next step will be to generate standardized factor scores for each joint and subject these to ANOVA by treatment.

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ASSOCIATION OF AN ASPARTIC-ACID REPEAT POLYMORPHISM IN ASPN WITH CONGENITAL DYSPLASIA OF THE HIP

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Purpose: Congenital dysplasia of the hip (CDH) is a common childhood skeletal disease, which is identified by abnormal seating of the femoral head in the acetabulum. Genetic factors play a considerable role in etiology of CDH. Asporin is an ECM protein which can bind to TGF-β1 and block its interaction with the TGF-β type II receptor, then sequentially inhibit the TGF-β/Smad signaling and TGF-β1 induced chondrogenesis. A genetic association of osteoarthritis (OA) and functional polymorphisms in the aspartic-acid (D) repeat of the asporin gene was identified in Japanese and replicated in several populations. As the inhibitory effect of asporin on TGF-β-dependent chondrogenesis, ASPN may be involved in pathogenesis of skeletal developmental abnormalities.