OARSI OSTEOARTHRITIS RESEARCH SOCIETY INTERNATIONAL

M01

Osteoarthritis in China and the USA: A Comparison of Prevalence and Risk Factors

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Background: Racial and geographic differences in the occurrence of disease have important public health implications and can provide clues about disease etiology. Little is known of the comparative prevalence of osteoarthritis (OA), although some studies have suggested low rates of hip OA in China. China, with its burgeoning elderly population will have an estimated 210 million people aged 65 and over by 2027. It is not known whether knee and hip OA, which affect over a quarter of people aged 55 and over in Western nations, will have a similar impact on the health of Chinese elders. To address these issues, we undertook a comparative prevalence and risk factor study of OA in Chinese and US Whites.

Methods: We surveyed a random sample of 2,517 (82% response) men and women (60%) in Beijing using questionnaires, x-ray methods and reading protocols from prior studies of OA in US Whites, which provided comparison samples: Framingham (knee, hand), SOF (hip) and NHANES I (hip). Standard definitions of x-ray OA used in all samples were K & L grade >=2 for OA of the knee and hand (any DIP, PIP, MCP or CMC-1) and for hip OA a minimum joint space <=1.5mm, osteophytes plus JSN, or 3 or more radiographic features.

Results: In women, x-ray and symptomatic (Sx) knee OA were 43-45% more common in Chinese than in US whites, but among men prevalence in Chinese and whites was similar. In Chinese, the lateral and medial compartments were involved with similar frequency, compared to a 5-7 fold ratio of medial to lateral involvement in Whites. Hip OA was rare in Chinese, with prevalences 80-90% less than in whites. X-ray hand OA was one-third, and Sx hand OA two-thirds, less prevalent in Chinese. Independent risk factors for knee OA in Chinese men and women include overweight, knee injury and heavy physical activity at work. Occupational squatting in men and nonoccupational squatting in both genders also increased the risk of knee OA in Chinese and accounted for some of the high prevalence in Chinese women.

Table. Crude prevalence of OA in Chinese ages >=65 and age-standardized prevalence ratios compared with same age US Whites

	Prevalence X-ray OA	Age-standardized prevalence ratio	Prevalence Sx OA	Age-standardized prevalence ratio
Knee-Women	46.6%	1.45 (1.31 – 1.60)	15.4%	1.43 (1.16 – 1.75)
Knee- Men	27.6%	0.90 (0.75 – 1.07)	7.1%	1.02 (0.70 – 1.42)
Hip - Women	0.4%	0.07 (0.01, 0.25)	0.1%	NA
Hip - Men	0.8%	0.19 (0.05, 0.49)	0.0%	NA
Hand - Women	47.0%	0.64 (0.59, 0.69)	5.8%	0.27 (0.21, 0.32)
Hand – Men	44.5%	0.67 (0.61, 0.73)	3.0%	0.31 (0.19, 0.69)

Conclusion: While hand and hip OA are uncommon in China compared to Western populations, knee OA is as, or more, prevalent in urban Chinese and is likely even more common in rural areas. Understanding the different patterns of OA prevalence in Chinese and Whites will likely enhance our appreciation of the causes of OA and may provide opportunities for prevention. Also, the burden of this disease in terms of pain and disability is high and millions of elderly Chinese are affected. The burden is likely to continue to grow as the population ages and obesity becomes more common.

M02

VALIDATION OF THE OARSI RESPONDER CRITERIA: RESPONDERS HAVE BETTER OVERALL HEALTH STATUS THAN NONRESPONDERS

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The OARSI response criteria were developed utilizing data from clinical trials of patients with osteoarthritis (OA) of the knee and hip (Osteoarthritis Cart 2000;8:395-403). They have not been tested in other data sets, nor have they been validated against other arthritis-specific or generic health status measures. We used data from an ongoing randomized 3-arm, 6 month trial of acupuncture in patients with OA of the knee to test the hypothesis that patients who fulfilled the OARSI responder criteria would have better overall health status as measured by arthritis-specific and generic health status measures. As of April 2002, 254 of 496 patients with symptomatic knee OA enrolled in this trial had finished 6 months of therapy and provided complete data for analysis. Patients were randomized to "true" acupuncture, "sham" acupuncture, or an education control group; data from all 3 arms of the trial were pooled for this analysis. Data from the WOMAC OA index, Health Assessment Questionnaire (HAQ), Medical Outcomes Study Short-form (SF) 36, European Quality of Life (EuroQoL) and Patient Global Assessment were available from entry and 6 months and were used in the analysis. Subjects were classified as responders or non-responders using "Proposition A" for knee oral NSAIDs with data from the WOMAC OA index. A total of 113 (45%) patients fulfilled the OARSI Responder Criteria. Comparison of the outcome measures at 6 months showed that there were highly statistically significant differences in favor of better arthritis-specific and overall general health status for responders compared with non-responders (see Table; $P < 0.001$ for all comparisons). These results provide strong evidence for the validity for the OARSI Responder Criteria, and support their use as outcome measures in OA clinical trials.

Outcome measure	Responders	Non-responders
WOMAC Total score	19.1 (11.0)	1.5 (15.8)
HAQ Pain score	0.77 (0.57)	1.41 (0.73)
HAQ Disability index	0.38 (0.34)	0.59 (0.45)
SF-36 Physical Function	61.8 (23.0)	38.1 (21.4)
SF-36 Role physical	71.1 (36.8)	6.9 (40.8)
SF-36 Bodily pain	66.7 (17.8)	66.7 (17.8)
SF-36 General health	75.9 (17.8)	64.2 (18.3)
EuroQoL Utility score	0.77 (0.15)	0.57 (0.25)
EuroQoL Health status	82.1 (13.8)	71.3 (17.1)
Patient global (0-5)	3.73 (0.80)	2.78 (0.85)

M03

CYCLOOXYGENASE INHIBITION: WHERE DO WE NEED TO GO?

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Soon after the discovery of the inducible isoform of cyclooxygenase (COX-2), the expectation emerged that the development of new agents that selectively inhibit COX-2 would optimize the analgesic and anti-inflammatory properties of the NSAIDs, while minimizing the potential for GI adverse events (mediated by inhibition of COX-1). The accumulation of clinical data from phase II and phase III trials, as well as in several recent large post-marketing outcome trials. support the concept that selective COX-2 inhibitors are associated with less GI toxicity than nonselective NSAIDs. However, it is now apparent that COX-2 is also expressed in a variety of noninflammatory tissues, including kidney, brain, neoplasms, bone, and cartilage, particularly under "physiologic stress" conditions. Therefore, it is not surprising that clinical studies indicate that selective COX-2 agents, like conventional NSAIDs, cause comparable rates of edema and hypertension, and may impair compensated renal function in the setting of congestive heart failure or volume depletion. Moreover, controversies have emerged, resulting from unanticipated outcomes of scientific investigations that have raised questions about the superior risk-to-benefit ratio of the coxibs compared to conventional NSAIDs. These include: 1) Do highly selective COX-2 inhibitors predispose to thrombotic events? 2) Does cardioprotective use of low dose aspirin abrogate the superior GI safety profile of the COX-2 selective inhibitors? 3) Are there differences among the coxibs with respect to effects on blood pressure and renal function? If so, are such effects reflective of the degree of COX-2 selectivity? It is likely that, through the variety of clinical and experimental studies in progress, we will have substantially greater insight into these question in the near future. As the issues are resolved, we will need to better understand the physico-chemical properties of coxibs and NSAIDs. It is probable that the overall safety profile of these agents can improve with drugs whose PK/PD profile favors shorter plasma half-life combined with accumulation at inflammatory sites. Whether the future of anti-inflammatory treatments will favor COX-2 selective coxibs, or whether combination therapies (i.e., with aspirin, a gastroprotective agent or an attached nitric oxide releasing moiety) will emerge as preferred agents will be determined by future studies.

M04

INTERLEUKIN-17 REGULATES THE LEVEL AND STABILITY OF CYCLOOXYGENASE-2 (COX-2) mRNA THROUGH SPECIFIC ACTIVATION OF THE p38 MITOGEN-ACTIVATED PROTEIN KINASE CASCADE IN TARGET CELLS: Role of AU-rich sequences in the 3' untranslated region (UTR) of COX-2 mRNA

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Interleukin-17 (IL-17) is acknowledged as a prototypic 1-cell-derived pro-inflammatory cytokine capable of activating inflammatory cells and found in abnormally high levels in the synovial fluid and joint tissues of arthritic patients. Our objective was to isolate the signaling pathways associated with IL-17-target gene expression in human chondrocytes (HC), synovial fibroblasts (HSF), and macrophages (HM). In preliminary work, we observed a rapid (5 mm), substantial (>15-fold), and sustained (>48h) increase in COX-2 mRNA, protein and PGE2 release following a rhIL-17 signal that was inhibited by SB202190, a selective, cell-permeable p38 MAP kinase inhibitor and a dominant negative construct of MEK-3. Western analysis using specific anti–phospho antibodies revealed that rhIL-17 increased specific phospho–rylation of kinase intermediates in the p38 MAP kinase pathway only, as well as the transcription factors CREB-1 and ATF-2. Co-transfection studies using chimeric CMV-driven constructs of Gal 4 DNA binding domains fused to the transactivation domain of ATF-2 or CREB-1 resulted in a 5.1 and a 2.7 fold increase in 5xGal-4 binding element– luciferase reporter activity following rhIL-17 incubations, respectively.

Transfection experiments with human COX-2 promoter constructs revealed a minor element of p38 MAP kinase-dependent transcriptional control after rhIL-17 stimulation. However, when HSF were activated with rhIL-17 for 6h (steady-state) followed by wash-out, the elevated levels of COX-2 mRNA declined rapidly (<2 h) to control levels. When rhIL-17 was added to fresh medium, COX-2 mRNA levels remained elevated for up to an additional 4-6 h; SB202190 compromised the stabilization of COX-2 mRNA by rhIL-17. Deletion analysis using transfected chimeric luciferase-COX-2 mkNA 3 '-UTR reporter constructs revealed that rhIL-17 increased reporter gene mRNA stability and translation via AU-containing distal regions of the UTR. This response was mediated entirely by a p38 MAP kinase/HSP27-dependent process. As such, IL-17 cell signaling is restricted to the p38 MAP kinase cascade involving transcriptional/posttranscriptional/translational control of target genes in the cell phenotypes tested.

M05

AZD3582: ANALGESIC EFFICACY AND GASTROINTESTINAL SAFETY IN RATS

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Aim of the study: To assess the analgesic, anti-inflammatory and gastrointestinal (GI)-sparing effects of AZD3582 , a COX-inhibiting nitric oxide donator (CINOD) being developed for the treatment of acute and chronic pain, after oral administration to rats.

Methods: AZD3582 and naproxen (0.30-100 µmol/kg) were administered to male Wistar or Sprague-Dawley rats and compared with vehicle. Effects on pain and inflammation were assessed in the carrageenan-induced monoarthritis and paw oedema rat models. GI-sparing effects were assessed after single and repeated b.i.d. dosing up to 18 days. Segments of the GI tract were examined macroscopically and haemorrhagic erosions were scored. Plasma samples were taken and analyzed to determine systemic exposure.

Results: AZD3582 and naproxen both caused dose-dependent reductions in carrageenan-induced paw oedema and pain behaviour. The anti-inflammatory effects were significant for both drugs at ≥10 µmol/kg, while the analgesic effects were significant for AZD3582 3µmol/kg at 3 and 5 h, and for naproxen 3 µmol/kg and 10 µmol/kg at 3 h and 5 h, respectively. Monitoring of plasma concentrations showed that the relative bioavailability of AZD3582 versus naproxen was 50-70% in the rat. Oral administration of AZD3582 markedly reduced acute and chronic gastric and intestinal damage compared to naproxen. Macroscopic examination of the GI tract revealed erosion and perforation of the small intestine in rats treated with naproxen at doses ≥60 µmol/kg. AZD3582 and vehicle did not cause any visible intestinal damage, except at the highest dose of AZD3582 (120 µmol/kg).

Conclusions: AZD3582 provides anti-inflammatory and analgesic efficacy in established rat models, which corresponds with previous results in a mouse model of analgesia, the acid-induced writhing model. AZD3582 has a low ulcerogenic potential and a favourable GI safety profile in rats compared with naproxen. The novel multi-pathway action of AZD3582, of balanced COX inhibition and nitric oxide donation, may provide a new strategy towards efficacious and GI-safe drugs.

M06

EFFECTS OF SC-560, SC-58125 & DICLOFENAC ON EXPRESSION & AND SYNTHESIZING ACTIVITY OF COX-1 AND COX-2 IN SYNOVITIS TISSUE IN OSTEOARTHRITIS

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Aim of the study: To investigate the relative contribution of the two COX isoenzymes COX-1 & COX-2 to prostaglandin release from human inflamed synovium in osteoarthritis (OA) in vitro and to furthermore determine and quantify the effects of COX-inhibitors on synovial gene expression of COX-1 and COX-2.

Methods: The effects of diclofenac, a COX-unspecific NSAID and of SC-560 and SC-58125, a selective COX-1 and COX-2 inhibitor, respectively, on the release of PGE2 & 6-keto-PGF1α from inflamed synovial tissue in vitro were compared. Tissue was obtained from OA patients during total joint replacement (n = 7). Tissue was incubated for 3 & 6 hours (37°C) at the presence of 0.1–10 µM diclofenac, SC-560 & SC-58125. Levels of PG were measured in the incubation media using ELISA. Expression of synovial COX-1/ COX-2 was determined by means of real time RT-PCR using the Light Cycler System.

Results: After 3 hours of incubation, untreated synovial tissue released 329 ± 106 ng PGE2/ g tissue wet weight (ww). 0.1/ 1/ 10 µM diclofenac caused the following inhibition (% inhibition vs. control, standard deviation not indicated) of PGE2 release: 49.6/ 59.9(*)/ 86.3(*) %. 44.4/ 78.8/ 85.3(*) % inhibition was seen for equimolar SC-560. Inhibitory potency of SC-58125 was 8.1/ 22.1/ 52.6(*) % (* $P< 0.05$ vs. control). After 6 hours of incubation, untreated synovial tissue released 1024 ± 314 ng PGE2/ g w.w. 0.1/ 1/ 10 µM diclofenac caused the following inhibition of PGE2 release: 74.5(*)/ 83(*)/ 97.4(*) %. 16.4/ 30.6/ 86.6(*) % inhibition was seen for SC-560. Inhibitory potency for SC-58125 was 15.4/ 62.8(*)/ 96.6(*) %. Comparable data were obtained for release of 6-keto-PGF1α. RT-PCR revealed a stable expression of COX-1 and COX-2 in the incubated synovial tissue compared to synovial expression of PBGD housekeeping gene.

Conclusion: Though loosing their specific COX-1/-2 selectivity at 10µM, SC-560/ SC-58125 show time-dependent increase of inhibitory potency during long-term incubation. The 1µM results most likely reflect COX selectivity, since 30.6 & 62.8 % inhibition for SC-560 & SC-58125, respectively, roughly add up to a total of 83 % inhibition seen for COX-unspecific diclofenac. At any concentration studied, this NSAID showed the strongest inhibitory potency. The significant participation of COX-1 in OA synovitis should be considered, weighing the potency of classical NSAID against the gastrointestinal safety of selective COX-2 inhibitors.

M07

A SUBGROUP ANALYSIS BY JOINT OF THE EFFICACY OF ROFECOXIB VERSUS NAPROXEN IN OSTEOARTHRITIS: FROM THE "ADVANTAGE" TRIAL

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We evaluated comparative osteoarthritis (OA) efficacy in the ADVANTAGE trial, a study examining the gastrointestinal (GI) tolerability of rofecoxib and naproxen in the treatment of patients with various primary sites of OA during a 12-week period. Patients meeting entry criteria (non-flare design) were randomized to receive rofecoxib 25 mg qd (n=2785) or naproxen 500 mg bid (n=2772). The primary endpoint was GI tolerability as defined by the incidence of discontinuations due to GI adverse experiences (AEs). OA efficacy was assessed in all patients by Patient Global Assessment of Disease Status (PGADS; a 0-100 mm visual analog scale), discontinuations due to lack of efficacy (LOE), and, in hand OA patients, also by the AUSCAN OA Hand Index, (three domains: pain, stiffness, and function scored on a 5-point Likert Scale). Baseline characteristics were similar between treatment groups. The primary sites of OA were: knee (50%), spine (24%), hand (16%), and hip (10%). The overall mean change in PGADS from baseline to Week 6 did not differ significantly between rofecoxib (-11.6 mm) and naproxen (-10.8 mm). Change in PGADS at Week 12 was also not significantly different between rofecoxib (-10.4 mm) and naproxen (-9.6 mm). Change in AUSCAN scores at Week 12 and discontinuations due to LOE did not differ significantly with rofecoxib versus naproxen. Between treatment efficacy similarity was maintained in subgroup analyses performed by joint based on the PGADS results. Significantly fewer (p=0.005) discontinuations due to GI AEs were observed with rofecoxib (5.9%) than naproxen (8.1%). In patients treated for three months, rofecoxib 25 mg qd demonstrated superior GI tolerability compared to naproxen 500 mg bid, while exhibiting a similar efficacy profile when assessing the entire cohort as well as across all primary sites of OA.

M08

INTEGRATIVE MEDICINE-A MATURING SCIENCE

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Until the last several decades,traditional medicine looked with disdain on so-called alternative/complementary medicines or non-medicinal approaches such as acupuncture and manipulation. Such alternative approaches were generally predicated on the basis of anecdotal input, with limited scientifically rigorous evaluation. The term "alternative" itself had a pejorative connotation, separating these therapeutic modalities (and their proponents!) from accepted allopathic management. Over the past decade, an increase in open-mindedness, combined with the acceptance that these alternative approaches required evidence-based support has led to a significant increase in interest in such approaches. Clinical studies are, however, impeded by the high cost of well-designed double-blind studies, and the fact that these therapeutic agents are generally non-patentable. It is important to separate out those approaches that are targeted to a specific disease, and agents intended primarily for "health maintenance"(usually the target of so-called nutraceuticals).The divisions between definitions of given agents as a pharmaceutical, nutraceutical or pharmanutraceutical have become blurred depending on the study design wherein they are tested. It is reassuring to observe

the high degree of scientific excellence being introduced to studies of alternative approaches. The present discussion will review agents which have been the subject of advanced investigation and attempt to develop a perspective of future applications. Integrative Medicine perhaps is a more fitting term for alternative,complementary medicine, allowing us to minimize past prejudices. The large number of Departments of Integrative Medicine at major schools of medicine attests to the maturing science in this field of study.

M09

Discovery and Evaluation of Potential Disease Modifying Anti-osteoarthritis Drugs Using Surface Plasmon Resonance Technology

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Aim of study: Pentosan polysulfates (CaPPS and NaPPS) and chondroitin sulfates (ChSs) have recently been shown to exhibit both symptom and disease modifying activities in osteoarthritis, but their mechanisms of action are still the subject of debate. Proteinases including human neutrophil elastase (HNE), which degrade the collagens and proteoglycans of the cartilage matrix in arthritic joints, could be targets for these drugs. In this study we compared the relative inhibitory and binding activity of these drugs to HNE and other cationic proteins.

Methods: The molecular interactions between NaPPS (of different origins), CaPPS, ChSs and some other sulfated polysaccharides with immobilized proteins were studied using a surface plasmon resonance (SPR) biosensor device (BIAcore2000). HNE or other cationic proteins (protamine, lysozyme and hyaluronidase) were covalently immobilized to a biosensor chip using amine coupling and the binding affinity of drugs was determined using published methods and a commercial software program.

Results: The binding activity obtained using SPR technology was compared with the drug inhibitory activity of HNE determined using a traditional functional assay. The results obtained by the two independent techniques showed good correlation and indicated that the degree and position of sulfate ring substitution were major determinants of HNE inhibition. Both methods afforded the following order of potency against HNE: ChSE > Heparin > CaPPS > NaPPS-B > NaPPS-N >ChSD > ChSA. Significantly, the SPR biosensor technology also demonstrated enzyme inhibitory differences between the CaPPS and NaPPS preparations of different synthetic origins.

Conclusions: The SPR technology offers not only a sensitive and reproducible means of ranking enzyme inhibitors as potential new drugs but also provides a bioassay for distinguishing between sulfated polysaccharides of different synthetic origins.

M10

MATRIX METALLOPROTEASE 1,3,13, NITRIC OXIDE AND PROSTAGLANDIN E2 PRODUCTION FROM OA CHONDROCYTES AND SYNOVIOCYTES STIMULATED BY INTERLEUKIN-1B SUPPRESSIVE EFFECTS OF GLUCOSAMINE-

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Aim of study: To evaluate the response of chondrocytes and synoviocytes to IL-1B in terms of MMP1,3,13, NO and PGE2 production, and the effects of glucosamine.

Methods: OA chondrocytes and synoviocytes were collected from 8 patients with knee OA during arthroplasty. Normal chondrocytes were collected from 7 patients with femoral neck fracture. Cells were isolated and culture cells were incubated with IL-1B ?and? 1,10,100 ?g/ml of glucosamine. After 24 hours, supernatant was collected to measure the levels of MMP1,3,13, NO and PGE2. As a control, dexamethasone and diclofenac were applied.

Results: The levels of all mediators except NO showed no difference between different cell types at base line. IL-1B stimulated the production of all mediators from chondrocytes and synoviocytes; however, the response of normal chondrocytes was low. After the stimulation, largest amount of mediators were produced in OA chondrocytes. During MMPs, the levels of MMP3 were highest; 100?g/ml of glucosamine suppressed all mediators from chondrocytes and MMP1 and 3 from synoviocytes.

Conclusion: Whereas all mediators tested are implicated in the pathogenesis of OA, MMP3 seems to contribute to it most. Glucosamine that has been used for the treatment of OA, may showed its effects by modulating the production of these mediators.

M11

TOPICAL GLUCOSAMINE AND CHONDROITIN SULFATE FOR OSTEOARTHRITIS OF THE KNEE: A RANDOMIZED CONTROLLED TRIAL

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Objective: Oral glucosamine has been reported to reduce the pain from osteoarthritis and may slow radiographic progression of OA of the knee. While oral glucosamine is readily absorbed, it is subject to first pass metabolism in the liver and uptake into non-joint tissues. Chondroitin sulfate is also known to be effective but the oral route is severely hampered by poor gastrointestinal bioavailability. This trial aims to assess the ability of a topical preparation of glucosamine sulfate and chondroitin sulfate to reduce pain related to osteoarthritis of the knee.

Methods: *Trial design:* Single centered, double-blind, randomized, placebo-controlled trial. Efficacy was assessed using a Visual Analogue Scale (VAS) for pain as well as the Western Ontario and McMaster Universities Osteoarthritis Index (WOMAC) and the SF-36 questionnaire

Intervention: Patients with painful OA of the knee were randomly assigned to use either a commercially available topical preparation containing glucosamine and chondroitin sulfate in addition to a small amount of camphor and peppermint oil or a placebo to be used "as required" for a period of 8 weeks.

Results: VAS scores indicated a greater mean reduction in pain for the active preparation group compared to the placebo group at both 4 weeks (mean change -2.6cm, SD 2.4cm vs -1.4cm, SD 2.4cm) and 8 weeks (mean change -3.4cm, SD 2.6cm vs -1.6cm, SD 2.7cm). After 4 weeks the difference between active and placebo groups in their mean reduction from baseline was 1.2 (95% CI: 0.1 to 2.4, p= 0.03) and this difference increased to 1.8 after 8 weeks (95% CI for difference between groups, 0.6cm to 2.9cm; P = 0.002). Based on data from daily diaries the VAS scores improved at a rate of -0.10cm per week (95% CI: -0.15cm to -0.05cm) in the placebo group and -0.20cm per week (95% CI: -0.25cm to -0.15cm) in the active group (p=0.005). The improvement in pain scores for subjects in the active group is supported by a trend towards improvement in the secondary outcome measures. Adverse events were infrequent, minor and equally distributed between the two groups.

Conclusions: Topical application of glucosamine and chondroitin sulfate is effective in relieving the pain from osteoarthritis of the knee and improvement is evident within 4 weeks.

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M12

Supplementary Vitamin E does not affect the loss of cartilage volume in knee Osteoarthritis: a 2 year double-blind randomized placebo controlled study

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Aim: To investigate the effect of Vitamin E supplementation on knee cartilage volume in subjects with osteoarthritis (OA) of the knee in a randomized, double blind, placebo controlled trial.

Methods: 136 subjects with knee OA (ACR clinical and radiographic criteria) were randomised to receive Vitamin E (500IU) or placebo for 2 years. Tibial cartilage volume was measured, using MRI, at the beginning and end of the study.

Results: Apart from there being more women in the Vitamin E group (42 (63%) vs 33(48%), baseline characteristics were similar in the 2 groups (67 Vitamin E, 69 placebo). One hundred and seventeen subjects (59 Vitamin E, 58 placebo) completed the study. Loss of medial and lateral tibial cartilage was similar in subjects treated with Vitamin E and placebo (Mean difference ± SD. Medial 157 ± 209μm^3 versus 187 ± 220μm^3 on placebo, p = 0.51. Lateral 186 ± 258μm^3 versus 251 ± 216μm^3, p = 0.19). There were no significant differences between the Vitamin E and placebo treated groups in improvement of symptoms from baseline. Dietary levels of anti-oxidants (Vitamin C, beta carotene, retinol equivalents) had no effect on cartilage volume loss.

Conclusions: Vitamin E does not appear to have a beneficial effect in the management of knee OA: it does not affect cartilage volume loss or symptoms. Further research is required into the possible role of supplementation of other dietary anti-oxidants in the treatment of knee OA.

M13

POST-GENOMIC ERA OF MEDICINE: FOLLOWING THE SCENT IN SEARCH OF THE ETIOLOGICAL GENE(S)

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Aim of Study: To identify differentially expressed mRNA and protein in normal and OA-affected cartilage using a "System Biology Approach to Genomics".

Methods: Various techniques such as Affymetrix Gene Chip Technology, SAGE, DD-PCR, highthroughput primary and subtracted cDNA library sequencing and proteomics on a bioinformatic platform have been utilized. More than 30,000 genes have been screened using these complementary methods. Validation of these targets was performed by Real Time PCR and proteomics. Six different buffers were tested for protein extracted from cartilage for proteomic analysis. One was selected after running a 2-D gel/LCMS of cartilage extract, chondrocytes ± IL-1 and synovial fluids. SNP analysis of DNA from normal and OA individuals was performed using a SYQENONE system. Functional and pharmacogenomics analysis of novel targets (for pharmacological intervention and gene therapy) were characterized in detail in human cartilage explants.

Results: Gene-mining efforts generated significant amounts of correlative data with OA. Approximately 1,500 transcripts were differentially expressed in normal and OA-affected cartilage (n=20-80). Using all the above technologies, cartilage and (OA)-disease specific dysfunctional transcripts were identified by microarray analysis. Among these, 71 were validated by Real Time PCR. 7% were always dysfunctional in all OA samples and 44% were found to be expressed in a variable (> 50%) fashion in OA-affected cartilage. Proteomic analysis of normal and OA-affected cartilage proteins, chondrocytes and synovial fluids identified known disease related protein spots and others not reported to be involved in the pathophysiology of OA. Pharmacogenomic analysis of differentially expressed genes with known "Anti-Rheumatic Drug" in vitro was used to curate the data. Functional genomic analysis was performed for some potential targets. These include TNFá convertase, IL-1 family receptors, Osteopontin, á5â1 and ávâ3 integrins, High Mobility Group-1 Protein and Cyclooxygenases.

Conclusion: Gene expression pattern of OA cartilage as compared to normal showed a complex "inflammatory and proliferation signature" representing various stages of chondrocyte ontology. In situ hybridization confirmed some of these targets. The post-genomic era of functional genomics (Functionomics) will need to narrow the bridge between correlative data and causative data. This will require a "System Biology Approach" integrating interactomes of interacting and interdependent disciplines forming a unified whole. Several proteins present in the synovium were identified in the cartilage (by proteomics) to demonstrate the influence of joint fluid in the pathophysiology of the disease. In summary, OA cartilage can be viewed as an inflamed/proliferative tissue, brimming with phylogistic products that could serve as targets for future pharmacological intervention. Thus, the dilemma, which conceptual framework shall we choose, osteoarthritis or osteoarthrosis? The implications are clear; exciting interventional 'anti-inflammatory' strategies for the former, pharmacological nihilism for the latter.

M14

ALTERED GENE EXPRESSION IN OSTEOBLAST-LIKE MG-63 CELLS SUBJECTED TO CYCUC HYDROSTATIC PRESSURE

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It is well known that bone cells normally function in a biomechanically active environment, and that an altered mechanical environment, such as in osteoarthritis, can contribute to bone remodelling. To better understand the relationships between bone cell metabolism and mechanical loading, the effect of intermittent cyclic hydrostatic pressure (HP) on mRNA levels for a subset of genes, potentially involved in bone remodeling was investigated. The influence of various loading protocols on the expression of 12 genes including Matrix Metalloproteinase-I and 3 (MMP-I, MMP–*3),* Collagen cxl (col I), and Osteocalcin (OC) was measured in the human osteoblast-like MG-63 cell line.

Methods: Cells were seeded into modified 6-well culture plates at 2 x I0[] cells per well, grown for 2 days in DMEM + 10% FCS media, transferred to serum free medium for 20 hours, then placed into the apparatus and subjected, at 37°C, to cyclic HP for one minute (0-0.8 MPa at 1Hz) then 14 minutes at rest, for 4, 8 and 12 hours. Control cells remained in serum free media, at 37°C, for corresponding times. RNA was extracted and RT-PCR performed using validated primer sets for assessing the 12 human mRNA species. Significance was established using a t-test with unequal variances. Experiments were performed in triplicate at least twice.

Results: Cyclic HP significantly increased ($p < 0.001$) mRNA for both MMP-1 and MMP-3 at all time points. The increase measured for MMP-1 was maximal after 4-8 hours, whereas MMP-3 increased further up to 12 hours. Levels of mRNA for Col I and OC were unchanged at 4 and 8 hours, but were significantly lower ($p < 0.001$) at 12 hours. mRNA levels for GAPDH and the other 7 genes were unchanged. These effects were not restricted to MG-63 cells, as similar changes in MMPs were detected in primary connective tissue cells, when subjected to the same conditions.

Conclusion: Cyclic HP significantly altered gene expression for some, but not all of the genes assessed. Previously, it has been shown that expression of both MMP-I and -3 is down regulated in explants of connective tissues under the same conditions. Altered gene expression in human osteoblast-like clonal cells under cyclic HP, further support a role for mechanobiology in the regulation of gene expression in conditions such as osteoarthritis.

M15

SUPPRESSION OF CHONDROCYTE-CHARACTERISTIC GENE EXPRESSION BY IL-1 IS MEDIATED THROUGH THE TRANSCRIPTION FACTOR C/EBP

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Cartilage-derived retinoic acid-sensitive protein (CD-RAP) and Type II collagen are secreted proteins expressed primarily by chondrocytes: the expression of both is repressed by interleukin 1β (IL-1β).

To investigate the transcriptional mechanism by which CD-RAP expression is suppressed by IL-1β, deletion constructs of the CD-RAP promoter were transfected into rat chondrocytes and incubated in the absence or presence of IL-1β.

The results revealed an IL-1β-responsive element located between –2138 and –2068 bp. As this element contains CAAT/enhancer-binding protein (C/EBP) motif, the function of C/EBPβ and C/EBPδ was examined. IL-1β stimulated the expression of C/EBPβ and δ and the direct binding of C/EBPβ to the C/EBP motif was confirmed by electrophoretic mobility shift assay. Expression of –2251 bp CD-RAP promoter construct was down-regulated by co-transfection with C/EBP expression vectors in a dose-dependent manner. Mutation of the C/EBP motif within –2251 bp construct abolished the inhibitory response to IL-1β.

These results suggest that C/EBP is a critical factor mediating IL-1β-induced repression of CD-RAP transcription. C/EBP expression vectors were also found to down-regulate the reporter construct containing the promoter and enhancer of the type II collagen gene. The IL-1β-induced repression of CD-RAP and type II collagen genes via stimulation of C/EBPβ and δ were confirmed in chondrocytes from normal human articular cartilage. Lastly, the enhancer factor, Sox9, known to be down regulated by IL-1, was shown to bind adjacent to the C/EBP site competing with C/EBP binding.

Taken together, these results suggest that C/EBPβ and δ play an important role in the IL-1β-induced repression of cartilage-specific proteins in joint disease and that expression of matrix proteins will be influenced by availability of both positive and negative trans-acting factors.

M16

DIFFERENTIALLY EXPRESSED GENES IN HUMAN ARTICULAR CARTILAGE

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Aim of study: The purpose of this study was to identify genes that are differentially expressed in cartilage during developmental, normal and osteoarthritic stages.

Methods: Total RNA from human fetal femoral, norma adult, mild and severe OA cartilage was extracted using TRIzol reagent. cDNA libraries were constructed into either λ ZAP Express vector or λ TriplEx2 vector through a PCR-based method. Phage plaques were randomly picked and positive inserts were identified by PCR. PCR products were then subjected to automated DNA sequencing and all generated sequences (expressed sequence tags or ESTs) were BLAST searched against the public databases.

Results: A total of 57,422 ESTs were obtained for gene expression profiling, including 13,398 ESTs from fetal, 17,151 ESTs from normal, 12,651 ESTs from mild and 14,222 ESTs from severe OA. Over 60% of the ESTs matched to known genes, and about 2% were putatively novel. Among the known gene matches, we identified 2,579 unique genes in fetal, 2,518 in normal, 1,938 in mild and 2,256 genes in severe OA cartilage. Relative EST frequency level was used to identify differentially expressed genes. In normal cartilage, as expected, collagen type II was the most abundantly expressed collagen. Decreases in COL2A1, 9A1, 9A3, 11A1, and 11A2 were observed in mild and severe OA. An increase in COL3A1 was noted in mild OA, while a dramatic increase was found in severe OA cartilage (Fig. 1). Moreover, mild and severe OA cartilage have higher expression levels of decorin (DCN) and lumican (LUM) compared to fetal and normal cartilage. Notably, proteoglycan 4 is elevated in mild OA, but decreased in severe OA when compared to normal cartilage (Fig. 2). More importantly, we observed higher expression of asporin in mild and severe OA compared to normal cartilage. Surprisingly, we found that CTGF was abundantly expressed in normal cartilage; however, its expression decreased in mild OA and dramatically decreased in severe OA (Fig. 3).

Conclusions: The EST-based approach is a powerful tool for large-scale identification of differentially expressed genes. To our knowledge, this is the first report where this approach was applied to four different types of human articular cartilage. Importantly, some of the identified genes are found to be associated with OA for the first time and could be of critical relevance to OA pathogenesis and progression. The knowledge will help guide us to identify disease pathways and eventually diagnostic biomarkers and/or therapeutic targets.

M17

OSTEOARTHRITIS in the biglycan/fibromodulin double deficient mouse is associated with increased level of cartilage oligomeric matrix protein and decreased levels of decorin and type II collagen in THE ARTICULAR CARTILAGE

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Aim: Mice deficient in biglycan and fibromodulin, two small leucine-rich proteoglycans, start to develop knee osteoarthritis (OA) by 2 months. During the progression of the disease, the articular cartilage lesions recapitulate the histological features of

human OA. In order to further validate the use of the double deficient mouse as an animal model of OA, an immunohistochemical characterization of the cartilage lesions was initiated.

Methods: Immunostaining for cartilage oligomeric matrix protein (COMP), decorin and type II collagen was performed on wild-type and double deficient knees. Two and 6 month-old knees, corresponding respectively to an early and an advanced stage of the disease in the double deficient mice, were analyzed.

Results: In two month-old wild–type knees, the expression of COMP was low and restricted to the extracellular matrix above the tidal mark. Comparatively, this area was more strongly stained in the two month-old double deficient knees. In addition, in the double deficient knees, the calcified matrix and its chondrocytes were also stained but at a lower level. At 6 months, the level of expression of COMP had increased in the wild-type mice but decreased in the double deficient mice.

Type II collagen stained the whole articular cartilage matrix and the chondrocytes in wild-type knees. In double deficient knees, type II collagen was not detected at the articular surface at 2 months, and by 6 months its absence had spread to the matrix surrounding the chondrocytes. Similar, but not identical, patterns of expression were observed for decorin.

Conclusions: The immunohistochemical results reported here are similar to immunostainings performed on samples from natural OA and other animal models. The transient increase in COMP level observed here has also been reported in Del1 mice, a transgenic model of OA and in natural OA in horses. Superficial loss of decorin occurs in human OA and the collagen loss pattern reported here mimics the degradation pattern of type II collagen in human OA. Taken together, our data support the use of the biglycan/fibromodulin double deficient mouse as an animal model of OA.

M18

LONG TERM HIGH-DOSE ASCORBIC ACID INTAKE IN THE HARTLEY GUINEA PIG INCREASES SPONTANEOUS OSTEOARTHRITIS SEVERITY

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Aim: To investigate the effects of 8 months' exposure to low, medium and high doses of ascorbic acid on the in vivo development of histological knee OA.

Methods: The Institutional Animal Care and Use Committee approved all aspects of this study. Forty-six male Hartley guinea pigs were fed diets with "low" (2.5-3 mg/day, n=13), "medium" (30 mg/day, n=14) and "high" dose (150 mg/day, n=16) from age 4 to 12 months. The low dose represents the minimum amount needed to prevent scurvy. The medium dose is the amount present in standard laboratory guinea pig chow. The high dose is the amount shown in previous studies to slow the progression of surgically induced OA in the guinea pig. Blood was collected at 4, 7, 10 and 12 months. Synovial fluid samples and knee joints were harvested at 12 months of age. Cartilage oligomeric matrix protein (COMP) was quantified in guinea pig synovial fluid by competitive ELISA assay with monoclonal antibody 12C4, which we found cross-reacted to guinea pig COMP. Hydroxyproline of cartilage, reflective of collagen content, was quantified by HPLC.

Results: Plasma analyses indicated successful dietary regulation of ascorbic acid levels . The mean total histological score was highest in the high dose group. When the total histology score for each animal was plotted against the mean plasma ascorbic acid level for that animal, there was a significant correlation (R=0.36, p=0.02). Synovial fluid COMP correlated with the severity of histologic disease (r=0.34, p=0.02) and increased with ascorbic acid dose ($p<0.001$ using test for linear trend). The mean/(SEM) hydroxyproline content of cartilage (mg/mg dry weight) increased across the dosage groups . Pairwise comparisons by ANOVA showed a significant increase in tissue hydroxyproline content in the high dose relative to the low dose animals ($p<0.05$).

Conclusions: Ascorbic acid increased cartilage collagen content in vivo, however, in contrast to surgically induced OA in the guinea pig, increased dietary intake of ascorbic acid worsened spontaneous OA.

M19

QUANTITATIVE *IN VIVO* MAGNETIC RESONANCE IMAGING TO ASSESS DISEASE MODIFICATION IN THE GUINEA PIG MODEL OF OSTEOARTHRITIS

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Aim of the study: To assess longitudinally, the effect of a metalloproteinase inhibitor (MPi) on joint integrity and cartilage destruction using *in vivo* magnetic resonance imaging (MRI).

Methods: Male, adult, Dunkin Hartley guinea pigs (9 months of age) were dosed for 66 days with vehicle (20% (v/v) DMSO, 60% (v/v) PEG400, n=16)) or 0.3mg/kg/day of a MPi (n=l0) via an osmotic mini-pump. Fat-suppressed MR images covering the entire left knee were acquired under halothane anaesthesia pre- and post-dosing using a 4.7T Varian 'Inova'. Three blinded segmenters used in-house software to determine cartilage volume on the medial tibial plateau (MTP) of the right knee. Histological assessment, using H&E stain, was performed on the left knee after fixation in 10% formalin and paraffin embedding. Indian ink staining was used to assess gross anatomical changes to the right tibial plateau.

Results: The animals remained healthy throughout the study. Similar weight gained ([]5%) was observed in each group. The vehicle dosed group lost 20.1±3.0% (mean±sem) of its MTP cartilage. This equates to a loss in cartilage volume of 0.47±0.08mm^3. Over the same period of time the MTP cartilage of the MPi dosed group increased by 0.28±0.04mm^3 or 16.8±3.0% ($P<0.001$, two-sided t-test). Visual assessment of the MR images indicated that the growth plate was closed prior to dosing, and the MTP size remained unchanged during the study. However, the cartilage MR signal intensity (water content) appeared brighter on the MTP in the MPi dosed group. This was not the case for the lateral tibial plateau where no difference was noted between the two treatment groups. Macroscopic assessment confirmed the MRI results: the area of damage on the MTP cartilage lesions in the vehicle group (23.8±3.8%) was markedly reduced after MPi administration (15.9±3.4%; $P<0.067$). Histological assessment indicated that the MTP cartilage from the MTPi dosed animal cartilage appeared 'normal'.

Conclusions: 3D MR image acquisition and segmentation allowed accurate *in vivo* quantification of MTP cartilage volume in a guinea pig model of osteoarthritis. Treatment with an MPi resulted in an unexpected increase in MTP cartilage volume. The likely explanation is that the MPi inhibited the degradative and not the reparative process. These data show that the disease modification effects of an MPi can be assessed using MRI.

M20

PROSTHESES SURFACE-ACTIVE PHOSPHOLIPID: DEFICIENCY IN OSTEOARTHRITIC JOINTS, USE TO REVERSE JOINT DEGENERATION IN HORSES AND ITS LUBRICATION OF IMPLANTED PROSTHESES

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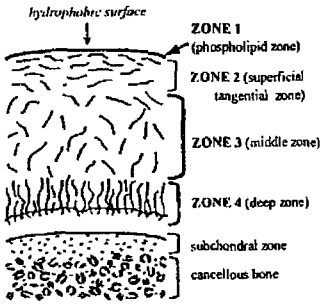
There is now much evidence to support the concept that the outermost hydrophobic lining layer of articular cartilage in the joint is not proteoglycan but an adsorbed oligolamellar layer of surface-active phospholipid (SAPL) which should now be recognised as Zone I - see diagram. This is also an excellent boundary lubricant which can reduce wear and friction under load to levels which are remarkable by engineering criteria.

We have just completed three studies, the first of which shows how SAPL is deficient in the cartilage surface of hips (10) and knees (8) replaced by prostheses at arthroplasty. In the second, in collaboration with orthopaedic surgeons, we have rinsed prosthetic hips and knees (35) retrieved at revision and have found each one to be coated with indigenous SAPL but in widely varying amounts.

In the third, in collaboration with veterinarians, we have completed up to 260 injections of synthetic SAPL into the joints of 160 horses and, in 80%, have been able to reduce or even reverse joint degeneration with 100% resolution of effusions.

This has led us to the theory that a deficiency of SAPL is a major causative factor in OA which can often be reversed by intra-articular Injections of exogenous SAPL. SAPL also lubricates prostheses whose failure might also be attributed to SAPL deficiency; this greatly reduces submicron wear debris in prosthetic hips.



M21

MRI AND RADIOLOGICAL FINDINGS

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Osteoarthritis (OA) is a group of clinically heterogeneous disorders unified by the pathological features of hyaline cartilage loss and subchondral bone reaction. Until about age 50, the prevalence of OA in women and men is similar. After that, OA is more prevalent, severe and generalised in women. The apparent increase in women presenting with polyarticular symptoms in middle age suggests a relationship between the onset of OA and the menopause. The trigger for the appearance of OA in middle-aged women is unknown but it has been suggested that it may be related to the hormonal changes occurring at the menopause. Whether these are active in all cases of OA in women, or are restricted to the so-called subtypes of OA ("menopausal OA" or "generalised OA") is unclear.

There has been increasing interest on the effect of oestrogen replacement therapy (ERT) on the incidence and prevalence of OA in post-menopausal women. A number of epidemiological studies examining the effect of ERT on the prevalence of OA have suggested a protective effect on knee and hip OA, but the results have not been statistically significant. Recent studies of incident knee OA have again suggested a risk reduction in women on ERT, but have failed to demonstrate statistical significance.

Women have less articular knee cartilage than men, matched for age, BMI and bone size. We have provided additional support for a potential benefit of ERT on OA. We showed that age-matched women taking ERT for more than 5 years had more articular tibial cartilage than women who had never taken ERT, independent of bone size, years since menopause, age of menopause, body mass index and physical activity. We speculate that maintenance of knee articular cartilage might be one mechanism by which ERT protects against knee OA.

M22

ESTROGEN REPLACEMENT THERAPY AND OSTEOARTHRITIS IN NON-HUMAN PRIMATES

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The reported effects of estrogen administration on severity of osteoarthritis (OA) in animal models are contradictory. Our group has focused on OA in cynomolgus macaques (*Macaca fascicularis*) as an animal model for the human disease, and we are particularly interested in determining the effects of estrogen replacement therapy (ERT) on this disease. Adult cynomolgus macaques develop OA naturally, the severity of which increases with age. Both the natural progression of the disease and the morphological appearance of the lesions are similar to OA in human beings. Thus, these animals provide a unique model for the study of naturally occurring OA. Cynomolgus macaques have a 28-day menstrual cycle, and intact animals have identical hormonal variations to women. Natural menopause occurs at an age that is close to the life expectancy of these animals; therefore, bilateral ovariectomy (surgical menopause) is used to model natural menopause. To examine the potential for a role of estrogen in this model, we examined adult monkey articular cartilage for the presence of estrogen receptors. Estrogen receptors were demonstrated using three different techniques (RT-PCR, immunoblotting, and immunohistochemistry) and also were shown in cultured chondrocytes to be functionally active.

We also have demonstrated that ERT in ovariectomized female monkeys results in significantly higher synovial fluid levels of IGF-1, IGF-2, and IGF binding proteins 1 and 3 compared with untreated ovariectomized monkeys, suggesting a potential stimulatory effect of estrogen on the IGF system in joint tissues *in vivo*. Furthermore, compared with untreated controls, IGFBP-2 production was significantly increased in conditioned media of chondrocytes cultured from monkeys that had received ERT *in vivo*, and this was associated with significantly greater sulfate incorporation.

Most recently, we have examined the effects of long-term ERT on the severity of OA of the knee joint in surgically postmenopausal female monkeys. This study included 166 adult female cynomolgus macaques that were bilaterally ovariectomized and randomly divided into three age- and weight-matched treatment groups for a three year period: 1) Premarin™-treated group, n=54; 2) soy phytoestrogen-treated group (n=60); and 3) control group (n=52). At necropsy, histological lesions of OA in the medial tibial plateau were graded, and cartilage and subchondral bone thicknesses and areas were measured. The data were summarized by principal components analysis and the resulting factors and individual

variables were compared using ANOVA and ANCOVA (age and weight as covariates). Cartilage lesions of OA were significantly less severe in the animals given ERT compared with those in the control group, and this treatment effect remained significant when adjusted for age and weight. The factor representing subchondral bone thickness/area was significantly higher, but the number of osteophytes was lower, in the ERT group compared with the control group. Soy phytoestrogen treatment had no significant effect on cartilage or bone lesions of OA.

These results demonstrate that estrogen has a role in OA and that ERT significantly reduces the severity of lesions of OA in this animal model of the disease, possibly through a stimulatory effect on the IGF system.

M23

HORMONAL MODULATION OF OSTEOCLAST ACTIVITY IN OA

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Osteoarthritis (OA) is accompanied by marked alterations in remodeling of the juxta-articular bone. These anatomic changes reflect local abnormalities in bone remodeling that lead to either increased (osteophytes, subchondral sclerosis) or decreased bone tissue (bone cysts). There is also evidence, at least during the early phases of OA, that there may be a decrease in juxta-articular bone mass. There remains considerable controversy regarding the mechanisms involved in the pathogenesis of these local disturbances in bone remodeling and their role in the progression of OA. The osteoclast is the principal cell type responsible for bone resorption under physiologic conditions.

Studies from our laboratory (as well as other groups) have provided considerable evidence implicating osteoclasts in the pathogenesis of focal juxta-articular bone erosions in rheumatoid arthritis (RA) and in animal models of inflammatory arthritis. However, there is little direct evidence that defines the role of the osteoclast in the remodeling changes associated with OA.

Recent studies have provided considerable insights into the role and mechanism of action of sex steroid hormones (including estrogens), as well as a variety of cytokines and growth factors, in regulating bone remodeling in both physiologic and pathologic conditions. Of interest, many of these same molecules also are involved in modulating chondrocyte function and remodeling of the cartilage matrix. Of particular interest is the recently described potent osteoclast-inducing factor, receptor activator of NF-?? ligand (RANKL). This ligand is a member of the tumor necrosis factor (TNF) family of cytokines and its activity is opposed by the naturally occurring inhibitor osteoprotegerin (OPG) which is a soluble receptor belonging to the TNF receptor family. Deletion of the RANKL gene in knock-out mice or blockade of RANKL with OPG in animal models of RA markedly reduces both focal and systemic bone loss. Of interest, there is also a marked attenuation of cartilage destruction, although studies thus far have not shown a direct effect of RANKL on chondrocyte function.

These results indicate that there is an important relationship between osteoclastic-mediated bone resorption and articular cartilage loss in inflammatory arthritis. Similar relationships likely apply to the progression of OA and insights into the underlying relationships between juxta-articular bone and cartilage remodeling could lead to new and innovative approaches in the treatment of OA.

M24

OVARIECTOMY INCREASES NITRIC OXIDE PRODUCTION IN OVINE PATELLAR CARTILAGE

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Aim: To determine the effect of oestrogen withdrawal by ovariectomy on cartilage nitric oxide (NO) production.

Methods: 12 adult sheep were used for this study, six of which were surgically ovariectomised (OVX). After euthanasia six months *post-op*, four 3mm discs of full-thickness cartilage were removed from the patella and cultured in Hams F12/l0% FCS for 24 hours. Media was then replaced (+100pg/ml ovine recombinant IL-lB in two of the wells) before incubation for a further 48 hours. NO release was assayed as conditioned-medium nitrite content (Greiss reaction) and corrected for explant DNA content. Similar cartilage discs were removed from the contralateral patella and immediately fixed in 10% neutral-buffered formalin. Immunostaining for iNOS (Cayman Chemicals) and nitrotyrosine (Upstate Biotechnology) was performed on 4µm sections following proteolytic epitope retrieval. Staining was quantified as the percentage of positively-stained cells, in the superficial and intermediate/deep zones.

Results: Ovariectomy significantly increased NO release by both resting (3.70±2.06 *vs* 1.26±0.35 nmol/µg DNA; p=0.005) and IL-lB-stimulated (5.65±2.12 *vs* 3.17±0.47 nmol/µg DNA; p<0.0001) cartilage explants. OVX sheep also showed an increase in the percentage of cells staining positively for iNOS and nitrotyrosine, which was significant in the intermediate/deep zone (Table 1).

Table 1: % of chondrocytes positively staining for iNOS and nitrotyrosine in the superficial (S) and intermediate/deep (I/D) zones

Stain:	iNOS		Nitrotyrosine	
Zone:	S	I/D	S	I/D
Control	46.7±22.3	11.2±19.7	54.9±16.2	29.9±15.6
OVX	69.3±17.9	45.7±20.3**	70.1±14.3	63.6±21.5*

*p=0.03;** p=0.008

Conclusion: In this study, OVX was found to significantly increase patella cartilage INOS expression and *ex vivo* NO release. While oestrogen is generally thought to be supportive of constitutive NO release, this and other studies suggest that oestrogen normally functions to suppress iNOS expression. Given the known deleterious effects of NO on chondrocytes, these data provide a possible mechanism for the enhanced progression of OA in postmenopausal women.

M25

REPRODUCIBILITY OF FIXED-FLEXION KNEE RADIOGRAPHY IN OSTEOARTHRITIC PATIENTS

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Aim of study: Radiographic joint space narrowing (JSN) is the primary structural endpoint for disease progression in OA clinical trials. Different knee positioning protocols have been proposed for this, including extended anteroposterior (AP), fluoroscopic semiflexed AP, semiflexed MTP PA, and fixed-flexion PA. Since the intra-articular distribution of cartilage loss in OA is heterogeneous,

the validity of JSN measurement with any of these depends on there being no change in the position of the femur relative to the tibia on serial examinations. Fluoroscopic semiflexed AP, semiflexed MTP PA and fixed-flexion PA fix the rotation and angulation of the tibia, but only fixed-flexion PA also fixes angulation of the femur. Most reports have focused on the alignment of the rims of the medial tibial plateau for reproducibility of radioanatomical positioning. In this study we examined the reproducibility of fixed-flexion radiographs acquired 1 year apart in terms of femoral angulation and knee rotation as well as tibial rim alignment.

Methods: Knee radiographs of 42 OA patients were acquired one year apart at a single center using the fixed-flexion technique and a Plexiglass positioning frame. Minimum JSW, tibial inter-rim distance, fibulo-tibial rotation, femoro-patellar rotation, femoro-patellar angulation and femoral intercondylar angulation were measured manually and independently by two specialized clinical-trials radiologists.

Results: The mean annual rate of JSN was 0.39 mm (SD = 0.75 mm) / 0.40 mm (0.84 mm), respectively for the two readers. Mean absolute change in tibial inter-rim distance between baseline and 1 year was 0.47 mm (0.53 mm) / 0.59 mm (0.66 mm). Mean absolute change was 1.22 mm (1.08 mm) / 1.18 mm (0.96 mm) for fibulo-tibial rotation, 1.95 mm (1.54 mm) / 1.97 mm (1.54 mm) for femoro-patellar rotation, 3.35 mm (2.74 mm) / 3.18 mm (2.88 mm) for femoro-patellar angulation, and 0.99 mm (1.02 mm) / 0.91 mm (0.85 mm) for femoral intercondylar angulation.

Conclusions: Fixed-flexion radiography shows reproducible positioning in terms not only of tibial angulation but also of rotation and femoral angulation.

M26

MAGNETIC RESONANCE IMAGING OF KNEE JOINT USING HIGH FIELD 8 TESLA SYSTEM

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Aim: The aims of this study were to 1) develop methods for the MR visualization of knee joint tissues using a whole body human 8 Tesla imaging scanner; 2) evaluate the quality and reproducibility of the high-resolution MR images *of ex vivo* encapsulated goat stifles; and 3) compare the high resolution 8 Tesla images with the conventional 1.5 Tesla images.

Method: Six intact, encapsulated goat stifles (right) were obtained from an abattoir. The *ex vivo* stifles were MR scanned within 24 hrs of necropsy. The 8T MR images of the stifles were obtained using a single strut cavity resonator and 2D spin echo sequence with MR parameters: FOV=l5cm, Matrix=1024x1024, ST=1 mm, TR=1000 msec; TE=20 msec; Scan time=10 mins; BW=l00 KHz. At 1.5T (GE, Signa LX), the MR images were acquired using 2D FSPGR with MR parameters: FOV=lOxlO cm, Matrix=512x256, ST=2 mm, TR=1500 msec; TE=20 msec; Scan time=12.5 mins; BW=31.25 Hz.

Results: The combination of high signal-to-noise ratio (SNR) and magnetic susceptibility of the high field MRI scanner provided an opportunity to visualize joint tissues with an unprecedented resolution. The inherent challenges of high field imaging, however, required elaborate techniques to produce homogeneous images. We have carried out imaging in RF coil in the coronal plane. This plane was in a triangular configuration with respect to the exciting source. As such, remarkably high quality images both in terms of resolution and contrast at 8T have been obtained. These images have enabled visualization of goat stifle anatomy namely, bone marrow, articular cartilage, anterior and posterior horn of menisci, femoral condyle, tibial plateau, patella, and patellar tendon. The 8T spin echo images were highly reproducible in all the scanned stifles. Using spin echo sequence, the SNR as well as delineation of the various joint tissue margins were better for 8T MR images compared to the conventional 1.5T.

Conclusions: The 8T MR images showed microscopic resolution of the fine details of the knee joint tissue microstructure such as intra-cartilage structure, edges of the meniscus, internal structure of ligament/tendon, and trabecular bone, compared to the images acquired using conventional technique at 1.5T. The 8T MRI has the potential to non-invasively visualize early changes and extent of lesion(s) in joint tissue structure, particularly cartilage, subchondral bone and bone marrow, in disorders such as osteoarthritis and rheumatoid arthritis.

M27

MUSCLE FORCE IN OSTEOARTHRITIS OF THE KNEE

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Aim: To quantify the difference in lower limb isometric force between patients with OA knee and their asymptomatic peers.

Methods: Mean isometric muscle force data collected on the most painful limb of 89 community dwelling older people aged 60-79 years with symptomatic OA knee referred for physiotherapy were compared with published reference data* on 106 asymptomatic gender-matched peers. For both the patient and the reference group, mean peak isometric force of 2 trials of a 5 second 'make' test was carried out with the participant seated, the foot free and the knee drawn by gravity into 90^{O} flexion.

Results: The majority of referred patients with OA knee had a lengthy symptomatic disease duration (60% > 5 years) and severe radiographic disease (55% < 2mm medial joint space width). Males with OA knee reported mean WOMAC pain and function scores# of 32 whilst females with OA knee reported a mean WOMAC pain and function scores of 40-42. These patients with OA knee could attain only a significantly lower 47-58% of knee extensor and 42-52% of knee flexor force generated by their age and sex matched peers (all $p<0.01$) (Table 1). The difference is even more marked when muscle force is expressed as a percentage of body weight and changes in pain and function with advancing age were more closely associated with this relative force measure compared with changes in absolute muscle force.

Table 1 Knee extensor and knee flexor isometric force Newtons (%body weight)

	Knee Extensors		Knee Flexors	
	OA	Reference	OA	Reference
Males				
60-69	195.6 (21%)	370.5 (48%)	106.6 (13%)	228.9 (30%)
70-79	173.1 (23%)	360.7 (48%)	97.4 (13%)	211.4 (28%)
Females				
60-69	147.7 (20%)	252.6 (39%)	79.6 (11%)	155.4 (24%)
70-79	106.8 (15%)	225.3 (38%)	58.5 (8%)	139.2 (23%)

Conclusions: The similar considerable decrease in knee extensor and knee flexor force demonstrated by people with established symptomatic OA knee suggests that preventative measures and rehabilitation should be directed at both muscle groups to optimally limit damaging impulsive loading and adduction moments on the knee joint during weight-bearing.

*Andrews RW et al.Phys Ther 1996;76:248-59.

WOMAC score range: 0 (no pain/difficulty)–100 (severe pain/difficulty)

M28

COMPARISON OF CARTILAGE MORPHOLOGY IN PROFESSIONAL WEIGHT-LIFTERS AND SPRINTERS WITH NORMAL VOLUNTEERS SUGGESTS THAT HUMAN ARTICULAR CARTILAGE CANNOT ADAPT TO MECHANCAL STIMULATION

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Aim of study: One of the most intriguing questions in musculoskeletal research is whether tissues are able to adapt postnatally to mechanical stimulation. Animal studies have produced inconsistent results regarding the functional adaptation of articular cartilage, but the development of quantitative MR imaging now permits to address this question directly in the human system. We tested the hypothesis that professional athletes with great muscle strength (weight lifters / sprinters) display higher cartilage thickness and/ or joint surface areas than normal volunteers.

Methods: We investigated 7 weight lifters (minimum Bavarian champion, maximum world champion), 7 bob sleigh co-drivers (minimum German champion, maximum world champion), and 14 normal volunteers, aged 18 to 35 years. Athletes had been starting training at ages 7 to 13, whereas volunteers had never performed training of muscle strength at all. Cartilage volume, thickness, and surface areas, and muscle cross-sectional areas (MCSAs) were quantified with MR imaging and dedicated software. Muscle strength was analyzed with a Cybex system.

Results: Weight lifters displayed significantly larger quadriceps CSAs (+ 30%, $p < 0.001$) and extending muscle strength (+26%, $p < 0.01$) than volunteers, and so did the bob-sleigh sprinters (+25%, $p < 0.001$ for CSA and +43%, $p < 0.001$ for strength). When averaging cartilage values over the entire knee, athletes displayed no significant differences in cartilage thickness and surface area. When analyzing single cartilage plates, there was a significant increase in thickness of patellar cartilage of the weight lifters (+ 13%, $p < 0.01$) and the sprinters (+16%, $p < 0.01$), but no significant differences in other parameters and/or other cartilage plates.

Conclusion: Patellar cartilage appears to be the only plate of the knee with some capacity for functional adaptation of its thickness. These data suggest that - opposite to muscle and bone - there is little (if any) room for postnatal adaptation of articular cartilage to mechanical loading.

M29

Modulation of Chondrocyte Phenotype in the Initiation and Progression of Osteoarthritis

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Whatever pleomorphism at first sight the cellular reaction pattern of chondrocytes during the osteoarthritic disease process display, they can be basically summarized in three categories. First, chondrocytes can activate or deactivate their anabolic or catabolic (matrix-degrading) activity by increasing or decreasing anabolic and catabolic gene expression. These changes have been in the focus of research activities for years now (i.e. synthesis of aggrecan and collagen type II as well as expression of e. g. various metalloproteinases). Secondly, chondrocytes can undergo phenotypic modulation implicating an overall severely altered gene expression profile of the cells in the diseased tissue. Thus, many cellular markers of fetal chondrocytes (e. .g cartilage collagen types IIA and X) are "newly" expressed in osteoarthritic tissue. Finally, the chondrocytes can undergo cell death, whether it is programmed (apoptosis) or not (necrosis); alternatively, they can proliferate to compensate for cell loss or to increase their synthetic activity, as cells do in many other tissues of the body as a reaction to tissue injury. It is a second issue which extracellular factors or circumstances initiate and promote these events and which are the intracellular mediators and genes involved in these processes.

Recent years have provided insights into all the areas addressed above, which are relevant to the initiation and progression of the osteoarthritic degeneration process though the exact sequence of the events remains unclear. An important step forward in osteoarthritis research was the introduction of functional genomic approaches into the research area. Functional genomics will in the future even more complement traditional biochemistry and molecular biology and will allow despite many limitations of this technology to paint "molecular portraits" of osteoarthritic chondrocytes vivo and in vitro.

M30

Proteolysis of type IX collagen and loss of biglycan, decorin and fibromodulin in progressive articular cartilage degradation

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Introduction: Type IX collagen and the leucine rich repeat proteoglycans (LRR-PGs) decorin, biglycan and fibromodulin are localised on the surface of type II fibrils and may provide steric protection. Removal of these surface molecules may be a prerequisite for type II collagenolysis. Using models of progressive articular cartilage degradation we studied the catabolism of aggrecan, types II and IX collagen, and the three LRR-PGs.

Methods: Articular cartilage from 3-6 month old pigs was cultured in DMEM ± 10µg/ml LPS or 1ng/ml IL-1alpha + 50ng/ml oncostatin M (IL-1/OSM). The glycosaminoglycan (GAG) and hydroxyproline (HyPro) content of the medium, extract and cartilage residue were measured. The proteoglycan and collagen fragments in the medium and extracts were separated by SDS-PAGE, and immunoblotted with specific antibodies.

Results: LPS and IL-1/OSM increased GAG release ($p < 0.0001$), with 60-80% of the total GAG released during week 1. There was no change in HyPro release in control cultures over the 4 weeks. LPS increased HyPro release during week 4 ($p = 0.02$), while IL-1/OSM increased collagen release during weeks 2, 3 & 4 ($p < 0.05$). IL-1/OSM induced a more marked collagenolysis with 60% of total collagen released after 4 weeks compared with 20% in LPS cultures. Immunoblotting with neoepitope antibodies demonstrated that the GAG release induced by both LPS and IL-1/OSM was due to increased aggrecanase and not MMP cleavage of aggrecan. During weeks 2-4, MMPs secondarily cleaved aggrecanase-generated G1 metabolites. The HyPro release induced by both agents resulted from collagenase cleavage of type II collagen.

Il-1/OSM and LPS induced loss of biglycan from the cartilage from week 1 and 2 onwards, respectively. This biglycan loss preceded the type II collagenolysis induced by both agents. There was a loss of decorin, fibromodulin and type IX collagen from the cartilage induced by both IL-1/OSM and LPS, which occurred concomitant with type II collagen degradation. There was little evidence of degradation of the LRR-PG core proteins in association with their release from the cartilage matrix. In contrast all of the released type IX collagen showed evidence of proteolysis with loss of the NC-4 domain.

Conclusions: Proteolysis of type II collagen fibrils is preceded by aggrecan and biglycan release form cartilage, while loss of decorin, fibromodulin and type IX collagen occurs coordinate with type II collagen catabolism. Studies utilizing proteinase inhibitors are now aimed at identifying the mechanisms/proteinaseas responsible for loss of the fibril associated molecules in progressive cartilage degeneration.

M31

MULTIPLE INTERACTING IL-1B-INDUCED TRANSCRIPTION FACTORS CONVERGE TO SUPPRESS CONSTITUTIVE *COL2A1* PROMOTER ACTIVITY

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Aim: IL-1b inhibits expression of the type II collagen gene (*COL2A1*) in chondrocytes following induction and activation of multiple transcription factors. Our goal was to determine whether these factors interact directly or indirectly with the *COL2A1* promoter.

Methods: Immortalized human chondrocytes (C-28/I2) were transiently transfected with deletion and mutation constructs in the pGL2 reporter vector containing *COL2A1* sequences spanning –577 to +3426 bp. The binding of nuclear factors in IL-1-treated cells or recombinant proteins to probes containing *COL2A1* elements or consensus sites was analyzed by EMSA. Cotransfections with expression vectors encoding wild-type or dominant-negative mutant proteins provided functional characterization.

Results: Inhibition by IL-1b was retained in the –577/+125 bp promoter region in the absence or presence of the 309 bp intronic enhancer and was associated with induction of Egr-1, NF-kB and ESE-1-binding activities in nuclear extracts. Egr-1 bound to sites within the –131 bp proximal promoter overlapping with Sp1 sites. ESE-1 bound to 2 sites within –403/–131 bp. In cotransfections, IL-1 was required for inhibition of promoter activity by pCMV-Egr1, whereas pCI-ESE1 by itself mimicked the IL-1 effect. Dominant-negative Egr-1, ESE-1 and srIkB vectors reversed the inhibition either completely or partially. However, NF-kB was found to act indirectly by inducing ESE-1 promoter activity and subsequent ESE-1 mRNA expression. Furthermore, constitutive Sox9, L-Sox5 and Sox6 mRNA levels were not suppressed by IL-1 in this culture model.

Conclusions: Our results show that Egr-1 acts by reducing the strong constitutive activity of the core *COL2A1* promoter by competing with Sp1 for binding and thus permits the direct effects of IL-1-induced transcription factors via sites distal to the proximal promoter. The rapid response observed is consistent with early cytokine-activated events that are usually associated with positive responses but produce a negative response in this promoter context by preventing interactions with constitutive factors. We conclude that the novel ETS factor, ESE-1, is the critical IL-1b-induced factor that directly suppresses *COL2A1* transcription and that it serves a repressor function via specific protein-DNA and protein-protein interactions involving both constitutive factors and other IL-1b-induced transcription factors.

M32

FACILITATED GLUCOSE TRANSPORT AND CHONDROCYTE ACTIVATION

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Glucose plays a pivotal role in chondrocyte differentiation, survival and extracellular matrix synthesis. Transmembranous glucose transport facilitated by glucose transporter proteins or GLUTs represents the first rate-limiting step in glucose metabolism. Recently, we demonstrated that four distinct GLUTs are detectable in human articular chondrocytes. These include cytokine-inducible GLUT1 and GLUT9, and constitutively expressed GLUT3 and GLUT8. IL-1B-accelerates glucose transport in chondrocytes, increases GLUT1 mRNA and protein expression as well as GLUT1 protein N-glycosylation and plasma membrane incorporation. The stimulatory effect of IL-1B on glucose transport in chondrocytes is mediated via cooperative interaction of protein kinase C and p38 MAP kinase pathways, and does not depend on nitric oxide and eicosanoid production. In contrast to IL-1B, TGFb1 - or IGF-1-increased glucose transport in chondrocytes is not associated with the up-regulated GLUT1 expression and is mediated via an Erk kinase-dependent mechanism. Expression of GLUT1 protein is markedly increased in human articular cartilage affected by osteoarthritis as compared to normal cartilage. Increased expression of GLUT1 is also detected in the articular cartilage from rabbit knees affected by experimentally-induced osteoarthritis. Inhibition of glucose transport in human chondrocytes with the glucose analogue 2-deoxyglucose suppresses GLUT1 membrane incorporation and inhibits chondrocyte activation by IL-1Bas measured by nitric oxide, IL-6, hexosaminidase and pro-MMP-1 production. Furthermore, pretreatment of bovine cartilage explants with 2-deoxyglucose completely prevents cartilage matrix degradation induced by IL-1. The anti-inflammatory effects of 2-deoxyglucose are reversible and do not depend on inhibition of glycolysis. Inhibition of glucose transport in chondrocytes also suppresses chondrocyte proliferation and glycosaminoglycan synthesis induced by TGFB1 and IGF-1. The 2-deoxyglucose effect is mediated in part via inhibition of Jnk, Erk and p38 MAP kinase phosphorylation. Results of this study demonstrate that facilitated glucose transport represents a novel mechanism regulating chondrocyte activation by cytokines and growth factors.

M33

BY INTERLEUKIN-1 VIA THE P38 MITOGEN-ACTIVATED PROTEIN SIGNALING PATHWAY IN AN ISOFORM-SPECIFIC MANNER MICROSOMAL PROSTAGLANDIN E SYNTHASE IS EXPRESSED IN HUMAN CHONDROCYTES UNDER REGULATION KINASE

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Aim: To investigate the expression and the regulation of the inducible microsomal prostaglandin E synthase (mPGES) in human chondrocytes.

Methods: Expressions of mRNA and protein mPGES were analyzed in cultured immortalized human costal chondrocytes (T/C-28a2 cell line) using northern blot and immunoblot, respectively. To investigate the effect of an inflammatory cytokine on these expressions, chondrocytes were stimulated by recombinant human IL-1beta (IL-1B) with or without pretreatment by various MAPK inhibitors. Levels of prostaglandin E2 (PGE2) secreted in culture medium after IL-1B stimulation were measured using EIA assay.

Results: IL-1B was found to upregulate the expression of mPGES both at mRNA and protein levels in a dose- and time-dependent manner which was in parallel with an increased secretion of PGE2. The p38 MAPK inhibitor SB203580 significantly suppressed IL-1B-induced PGE2 synthesis and IL-1B-induced mPGES expression. However, specific inhibitors for the alpha isoform (SC906 and SC335A) of p38 MAPK did not exhibit this inhibitory effect on mPGES expression, suggesting that, in chondrocytes, IL-1B triggers the expression of mPGES via a non-alpha-p38 MAPK pathway.

Conclusions: In human chondrocytes, IL-1B induces mPGES, mainly through a p38 MAPK signaling pathway, in an isoform-specific manner. The mPGES enzyme could be considered as a novel therapeutic target in order to block the catabolic and inflammatory properties of PGE2 in cartilage.

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M34

Articular chondrocyte proliferation and cyclin D expression

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Aims: Cell proliferation is critical to tissue homeostsis and repair. Our aims were to identify the critical D type cyclins (CD) that control articular chondrocyte (AC) proliferation, identify mechanisms that control expression of these cyclins, assess the effects of of CD1 over expression in ACs.

Methods: **1.** D type cyclin expression profiles were determined in total RNA samples isolated from human AC samples and isolated chondrocytes obtained from total joint replacement patients, using semi-quantitative RT-PCR. **2.** Changes in expression of the D type cyclins under differing in vitro conditions (monolayer vs suspension, +/- FBS) were assessed by Northern and Western blot analyses. **3.** Effect of DNA methylation status on intrinsic cyclin D expression was assessed by 5-azacytidine (5-AZA) treatment. **4.** Proliferative effects of CD1 over expression was assessed in bovine ACs using a CD1-expressing adenovirus.

Results: **1.** Of the three D type cyclins, CD2 was constitutively expressed in all human cartilage samples, CD1 expression was variable and was linked to the expression of the proliferative marker cyclin A and the CDK inhibitor, p21. Further, CD1 expression was correlated with overt matrix damage. CD3 was not expressed by ACs. **2.** In bovine ACs cultured as serum-free, non-adherent aggregates, CD1 expression was substantially down regulated over time, reaching minimally detectable levels by Day 14. CD1 expression was responsive to mitogens (FBS, bFGF), but the mitogenic response was also lost over time in culture. **3.** Treatment of bovine ACs with 5-AZA for three days resulted in dose-dependent increases in steady-state expression of both cyclin D1 and p21. **4.** Cyclin D1 over expression did stimulate some chondrocyte proliferation, however, this response was transient (1-4 days) and was substantially less than the response of these cells to bFGF treatment. Significantly, adenoviral CD1 expression was not accompanied by increased p21 expression, whereas bFGF up regulated expression of both genes.

Conclusions: CD1 is the primary regulated D type cyclin in ACs. Expression of CD1 is dependent on both substrate provision and exposure to mitogens. CD1 expression, and AC proliferation, is minimal in conditions that support the fully differentiated articular chondrocytic phenotype. Repression of CD1 expression is mediated, at least in part, by DNA methylation status. CD1 expression, by itself, is insufficient to stimulated chondrocyte proliferation. Co-expression of the CDK inhibitor/adaptor molecule p21 may also be necessary for the activation of G1 phase CDKs and consequent chondrocyte proliferation.

M35

Suppression of cell death and OA progression by inhibiting intracellular p38 MAP Kinase in chondrocytes: in vitro and in vivo evidence

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Introduction: Chondrocytes are the only cells in articular cartilage, and thus responsible for its structural integrity by maintaining its extracellular matrix. Osteoarthritis (OA) is characterized by the loss of chondrocytes and destruction of extracellular matrix in cartilage, increase of subchondral bone density, and formation of osteophytes. However, the intracellular pathway that leads to cell death and OA progression is not clear. We test the hypothesis that p38 MAP kinase, a stress kinase activated by pro-inflammatory cytokines and mechanical stress, plays an important role in regulating chondrocyte death and OA progression.

Material and Methods: For in vitro study, human OA chondrocytes were isolated from replaced joint cartilage. Anti-Fas mAb was used to induce cell death, and SB203580 was used to inhibit p38 MAPK activity. Apoptosis rate was quantified by flow cytometry of annexin-V positively labeled cells. Activities of p38 MAPK and ATF-2 were determined by western blot. For in vivo study, three lines of transgenic mice were generated in which a dominant negative (DN) p38 MAPK transgene was expressed in cartilage under collagen II promoter. The joints of transgenic mice were analyzed by X-ray radiograph periodically during aging.

Results: Treatment of OA chondrocytes in vitro with p38 inhibitor blocked not only anti-Fas induced apoptosis, but also total cell death. Concurrently, chondrocyte proliferation was increased. Activity of p38 kinase was up-regulated by induction of apoptosis with anti-Fas, and down regulated by inhibition of apoptosis by SB203580. Induction of chondrocyte apoptosis resulted in the increase of the activity of caspase-3 and ATF-2, a substrate of p38 MAPK. Suppression of p38 activity completely inhibited the activity of these factors. In vivo, complete suppression of p38 MAPK activity in chondrocytes (DNp38 homozygotes) led to the lack of endochondral bone during development, however, partial suppression of p38 MAPK in cartilage (DNp38 heterozygotes) inhibited OA associated symptoms during aging, including the increase of subchondral bone density and formation of osteophytes.

Discussion: Our study suggests p38 MAPK activity is a key regulator of cell death and OA progression in cartilage. Repression of p38 activity inhibits chondrocyte apoptosis by depressing ATF-2 and caspase-3 activity, while elevation of p38 induces apoptosis by stimulating their activity. Thus, inhibition of p38 MAPK activity blocks the loss of cells in cartilage by decreasing apoptosis and enhancing cell proliferation. In vivo data demonstrate that inhibition of p38 MAPK in chondrocytes leads to suppression of OA progression. Thus, p38 MAPK pathway is of potential therapeutic importance as a target for the prevention or treatment of OA.

M36

ADENOVIRAL OVEREXPRESSION OF TGF-β AND BMP INHIBITORS DURING EXPERIMENTAL OSTEOARTHRITIS

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The aim of this study was to assess adenoviral overexpression of TGF-β and BMP inhibitors on early osteophyte formation, fibrosis and cartilage proteoglycan (PG) content during experimental osteoarthritis (OA).

Methods: Adenoviruses expressing the intracellular BMP inhibitor Smad6 (Ad-Smad6), the intracellular TGF-β inhibitor Smad7 (Ad-Smad7) or luciferase as a control (Ad-Luc) were used. On day 1, adenoviruses (pfu $1\text{-}10^7$) were injected in right knee joints of C57Bl/6 mice to transfect the synovial lining. One day later, papain (1U) was injected i.a. to induce experimental OA. After 7 days, knee joints were isolated and processed for routine histology. Osteophyte size, fibrosis and cartilage PG content of the lateral and medial femur/tibia plateau were all measured by a blinded observer using a computer aided image analysis system.

Results: Papain injection caused the formation of osteophytes primarily on two locations; the lateral and medial femur near the margins of the articular cartilage. Despite a reduction in osteophyte size in the Ad-Smad6 group compared to Ad-Luc, this did not reach statistical significance. In contrast, Smad7 overexpression significantly decreased osteophyte formation on both locations by ~50% and ~60%, respectively, compared to Ad-Luc. Moreover, fibrosis induced by papain injection was significantly reduced by Smad7, but not by Smad6 overexpression, compared to Ad-Luc. Strikingly, although overexpression of Smad6 and -7 was restricted to the lining, Smad6 increased cartilage PG loss in all articular surfaces in the order of 30-40%, compared to the Ad-Luc group. Smad7 overexpression also increased PG loss, although the overall effect was smaller than after Smad6 overexpression.

Conclusions: Since Smad7 and not Smad6 reduced osteophyte size, the major contributor to early osteophyte formation is most likely TGF-β. TGF-β also seems the primary factor responsible for fibrogenesis during experimental OA. Interestingly, inhibition of the TGF-β/BMP signaling pathway in the lining affected cartilage PG content. Most likely, inhibition of TGF-β/BMP signaling leads to altered growth factor expression in the lining, which results in enhanced cartilage PG loss.

M37

Difference in early expression of *c-fos* gene via increase of intracellular Ca^{2+} ion reflects repair potentials of superficial defects on articular cartilage in fetal and adult rats

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Aim of Study: Superficial defects in adult articular cartilage do not heal, but similar injuries in the fetal knee heal spontaneously. To clarify the early process of healing, we investigated the relationships among the repair capacity, early induction of *c-fos* gene expression and intracellular Ca^{2+} ion (i) after the injury in adult and fetal rat articular cartilage.

Methods: Partial defects of 150 to 200μm-deep were created on the surfaces of the knees of adults and E19 fetuses. The repair process from 1 to 48 hours after the operation was histologically observed, and the expression of *c-fos* mRNA was analyzed using RT-PCR and in situ hybridization. Chondrocytes were isolated from 5-week-old rat joints and cultured. The *c-fos* expression after application of purinergic agonist ATP and Ca^{2+} ionophore A23187 was measured. The effects of ATP on i was observed using fura-2 fluorescence image.

Results: The defects in the fetuses were almost repaired within 48 hours, but that did not occur in adults. In fetuses, the *c-fos* gene expressed in chondrocytes just around the wound. It started 1 hour after the injury and continued for 6 hours. The region expressing *c-fos* expanded on the surface of cartilage. In contrast, no obvious *c-fos* expression was observed in adults, for up to 48 hours. ATP and A23187 elicited the immediate expression of *c-fos* in primary cultured chondrocytes. When ATP was applied to cartilage slices, fetal chondrocytes showed transient increase of i, whereas adult cells did not display the response.

Conclusions: The distinct expression patterns of *c-fos* after cartilage injury may reflect the repair potentials of fetal and adult articular cartilage. The ATP responses of cultured chondrocytes and fetal cartilage suggested a process that the increase of i by the stimulation of purinergic receptor induced the expression of *c-fos*. This process may work only in the repair of injured fetal cartilage.

M38

PREDICTORS FOR THE INCIDENCE AND PROGRESSION OF LUMBAR SPINE INTERVERTEBRAL DISC DEGENERATION: THE CHINGFORD STUDY

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Aim: Incidence and progression of the individual radiographic features of lumbar spine disc degeneration have not previously been studied. We therefore examined for the rates and predictors of radiographic incidence and progression of lumbar spine disc degeneration in a population-based cohort of women.

Methods: 796 paired lateral lumbar spine x-rays taken at entry to the study and at year 9 were examined. The films were read by one experienced reader for the individual features of anterior osteophytes (AO) and disc space narrowing (DSN) using the Lane grading score and an overall Kellgren and Lawrence grade (KL) at each lumbar disc space L1-5. Prevalent cases were defined as the presence of KL 2+ or a Lane grade of 1+ or 2+ for either AO or DSN in at least one of the four disc spaces. Incident cases were defined as < grade 2+ at year 1 and grade 2+ at year 9. Progression cases were defined as grade 1+ at year 1 and a change to a higher grade over the 9 years. Predictors of incidence and progression including age, body mass index (BMI), smoking, ERT, physical activity, back pain, presence of OA at the hip, hand and knee were examined using logistic regression. In the analysis adjustments were made for age and BMI.

Results: At entry the mean age was 53.8 ± 6 years and BMI 25.4 ± 4.0 kg/m^2. There were 19% of new cases defined by AO, and 16% by DSN, giving an average incident rate of 2% per year. Progression occurred in 25% of cases defined by AO and 34% defined by DSN. Incident DSN was associated with age, BMI, back pain (p= 0.02) and baseline AO2+. Progression of DSN was associated with BMI, knee osteoarthritis, baseline AO2+ and back pain (p <0.001). Incident AO was associated with age and BMI. Progression of AO was associated with age, BMI and baseline DSN2+.

Conclusions: This is the first population study of the rates and risk factors for the incidence and progression of the individual features of AO and DSN of lumbar spine disc degeneration. Risk factors include age, BMI, back pain, baseline individual features and osteoarthritis at other sites. These factors appear to be site and feature specific.

M39

CHONDROCYTES OR STEM CELLS?

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Given the poor reparative abilities of articular cartilage, many attempts to use cell-based therapies to augment or replace cartilage have been made. There is an ongoing scientific debate regarding the use of stem cell versus chondrocyte-based therapies for articular cartilage repair/regeneration. It is presently unclear whether either strategy is useful for the treating cartilage loss due to either trauma or degeneration.

There are many sources of stem cells and several sources of chondrocytes that could be used for cartilage repair or regeneration. Many types have been tried, albeit with most strategies concentrating on isolated chondral or osteochondral defects, in both the research and clinical settings. Cell-based therapies for degenerative conditions such as osteoarthritis represent considerably greater challenges, for which little research has been done.

An issue that is often raised concerning mesenchymal stem cells is the fact that they will differentiate into hypertrophic chondrocytes and die by apoptosis in both *in vitro* and *in vivo* systems that facilitate chondrogenesis. Thus, it is argued that these cells may not give rise to articular chondrocytes. However, this may be a limitation of the systems used, since prevention of the hypertrophy of these cells can be accomplished in the appropriate conditions.

There are issues related to the commercial possibilities for cell-based therapies. The time and costs associated with any type of autograft technique that involves *in vitro* manipulation outside of the operating room do not make these techniques commercially attractive. Our experience with creating tissue-engineered stem cell cartilage constructs has shown us the difficulties with this strategy. Although many companies are interested in such techniques, allograft or xenograft cell therapies that might be made into appropriate "off-the-shelf" products are more desirable. This is true whether one is considering the use of either stem cells or chondrocytes.

Clearly, the stimulation of cells *in vivo*, or at least within the operating room setting, is the most desirable therapeutic option. This could still involve either chondrocytes or mesenchymal stem cells, since sources of both exist close to or within the joint. For chondrocytes, the concern is the low cell number available for stimulation in joints with larger defects or more degenerated cartilage. For stem cells, the synovium or subchondral bone marrow are readily accessible sources, as ours and other work demonstrates. Recent reports of cells with stem cell-like characteristics in the superficial zone of cartilage itself offer another potential source of reparative cells. All of these cells may be amenable to stimulation with appropriate factors that stimulate chondrogenesis and cartilage extracellular matrix production. However, such strategies would have to include proliferation at an early stage, and thus become quite complex to orchestrate.

In summary, the answer to the question posed in the title depends on the strategy one is pursuing. It is unclear which strategy will succeed most effectively in cartilage repair and regeneration. However, it is obvious that more than one strategy will be required for the many different conditions encountered in joint pathology.

M40

HOW IMPORTANT IS THE BIOMATERIAL/SCAFFOLD FOR CARTILAGE REGENERATION?

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Articular cartilage can tolerate a tremendous amount of intense and repetitive physiological stress, but it also exhibits a striking inability to heal. Rapid, orderly and mechanically competent restoration of articular cartilage remains a challenge, and it is an open question whether cartilaginous tissue constructs should be engineered in vitro, or whether cartilage regeneration should be carried out in vivo. In either case, it is critical that cartilage transplant contains biosynthetically active cells within a three-dimensional biomaterial scaffold.

A biomaterial scaffolds for functional tissue engineering is generally designed to provide a structural template for tissue development, to facilitate transport of nutrients, oxygen and regulatory molecules to and from the cells, and to biodegrade in parallel with the accumulation of tissue components. Scaffolds investigated to date vary with respect to material chemistry (e.g., collagen, synthetic polymers), geometry (e.g., gel, fibrous mesh, porous sponge), structure (e.g., porosity, pore size distribution, orientation and connectivity of the polymer phase), mechanical properties (e.g., compressive stiffness, elasticity) and degradation (e.g., degradability, degradation rate, degradation products). Scaffold chemistry is important because each scaffold is also an informational template providing specific signals to the cells, by itself as well as by immobilized growth factors and enzymes. Scaffold structure is also important as it determines the cell attachment and function, the transport of chemical species, and in conjunction with scaffold chemistry the course of scaffold degradation. Scaffolds should be made of biocompatible, biodegradable materials to minimize immunogeneicity in vivo. The rate of scaffold degradation should ideally match the rate of synthesis and functional assembly of the extracellular matrix, and this rate can depend on scaffold material and structure, spatial density and metabolic activity of attached cells. The maintenance of mechanical properties of the scaffold over the course of tissue regeneration may be critical for its efficacy, and the scaffold can modulate the stress-strain environment at the cellular and tissue levels.

This contribution will discuss the general and specific requirements for biomaterial scaffolds used for functional tissue engineering of cartilage.

M41

REPAIR AND REGENERATION OF ARTICULAR CARTILAGE POTENTIAL APPLICATIONS IN OSTEOARTHRITIS EVAULATION OF OUTCOMES

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With the intention of relieving pain and improving mobility for people suffering from osteoarthritis, surgeons and scientists have developed a variety of approaches to restoring cartilaginous articular surfaces. These approaches fall into two categories: 1) stimulating formation of new cartilaginous tissue, 2) transplanting chondral and osteochondral allografts or autografts. Recent work has combined these two approaches to create grafts, pre-formed in vitro and consisting of matrices containing chondrocytes or stem cells, that can be implanted in synovial joints. Experimental studies have shown that multiple variations of these approaches will restore some form of cartilaginous articular surface in animal joints, but successful formation or transplantation of cartilaginous tissues in animals with experimentally created joint surface defects does not prove that a given method has the potential to relieve joint symptoms or improve joint function in humans with osteoarthritis. The information developed form clinical studies thus far is not sufficient to support the use of any of these approaches in patients with osteoarthritis. Yet, the effort to restore cartilaginous articular surfaces has shown enough promise that investigators should now evaluate the results of methods of restoring cartilaginous articular surfaces in ways that have the potential to identify the most promising approaches to the treatment of defined clinical problems. Important issues concerning experimental models include the type of articular surface defects studied, the age of the animals and differences in joints and articular cartilages among species. Important considerations in assessing the outcome of procedures designed to restore an articular surface in humans and animals include the overall function of the individual animal or patient, the function of the specific joint, the structure of the joint and the structure, composition and mechanical properties of the new tissue. This rigorous comprehensive approach to evaluating methods of restoring cartilaginous articular surfaces is based on the principle with the goal of any of these methods is sustained improvement in joint function and decreased joint symptoms in people with traumatic or degenerative joint damage. It is important to appreciate that tissues that differ from normal articular cartilage in structure, biochemical composition and biomechanical properties may have the potential to function well as an articular surface despite the fact that they fail to restore a tissue that is identical to normal articular cartilage.

M42

Repair of Articular Cartilage Lesions with PolyActive™ 70/30 and PolyActive 55/45 in Rabbits: with and without Allogeneic Chondrocytes

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Introduction: The aim of our study was to assess the performance of biocompatible and biodegradable PolyActive™scaffolds (PA), polyethylene glycol terephthalate – polybutylene terephthalate (PEGT-PBT) block copolymers, without or with allogeneic chondrocytes in cartilage defects in New Zealand White rabbits.

Materials and Methods: PA70/30 and PA55/45 scaffolds were implanted in 4 mm diameter 'fresh' and 'old' cartilage lesions in 22 female NZW rabbits. The rabbit knees were randomly divided into a control group, a sham group, a PA70/30 group and a PA55/45 group. An osteochondral defect was drilled in the knees of 6-month old rabbits and then filled with PolyActive™or left empty (control). The follow-up was three months. In the second study the knees of 14 6-month-old rabbits were randomly divided into four groups. 'Fresh' osteochondral defects were left empty (i); treated with an empty scaffold (PA55/45) (ii); treated with a PA55/45 scaffold seeded with primary, allogeneic rabbit chondrocytes (iii); or treated with a scaffold with seeded primary, allogeneic rabbit chondrocytes which were subsequently cultured (iiii). After sacrifice the femoral condyles were prepared for histology. Sections were scored using the histological O'Driscoll score for cartilage repair.

Results: In general the defects were filled with fibro-cartilaginous tissue on top of incompletely recovered subchondral bone. Interestingly, 88% of the tissues within the PolyActive™ scaffolds, was bonded to the adjacent cartilage to at least one side of the graft. From the empty PA55/45 scaffolds in 'fresh' defects, all tissues in the constructs were bonded to the adjacent cartilage. In the first study PA70/30 resulted in an overall significantly better repair tissue than PA55/45, if implanted in a fresh osteochondral defect. Conspicuously, the PA70/30 scaffolds were surrounded by a large number of giant cells and phagocytes, suggesting an extensive ongoing degradation process, which probably inhibits the bone formation.

Conclusion: Seeding, or seeding and culturing with allogeneic chondrocytes did not improve cartilage formation in this model.

M43

MECHANISM OF PAIN REDUCTION BY HYALURONAN DERIVATIVES (HYLANS)

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We explored the possibility that intra-articular hyaluronan solutions act as elastoviscous filters for the transmission of force to mechanosensory channels of pain receptors (nociceptors) and that this effect depends on the molecular weight of the hyaluronan solutions.

Methods: Electrical activity of single fibers innervating the intact knee joint was recorded from fine filaments of the median articular nerve of anesthetized rats. Mechanical stimulation consisted in controlled inward and outward rotations of the knee joint within and outside its normal working range, for 50 s every 5 mm. Solutions of sodium hyaluronan of different molecular weights (~2000,000 to ~6,000,000) were injected into the joint cavity (0.05 ml). Impulse discharges evoked by the movements were compared before and after injection. Also, patch-clamp recordings in the cell-attached and outside-out configurations were performed from *Xenopus* oocytes in Barth's medium (control condition) and exposed to hylans of different elastoviscosities. For mechanical stimulation graded suction was applied through the microelectrode.

Results: Hylan G-F 20, a clinically used hyaluronan derivative (MW —6M), significantly reduced in the course of 1 hour the number of nerve impulses evoked by movements in the rat joint pain fibers to about 60 % of the control frequency. No significant decreases of the mean impulse frequency were obtained with lower MW (<2 M) hyaluronan solutions used therapeutically. In mechanosensory channels of intact oocytes and of outside out membrane patches, suction-evoked channel activity was significantly reduced in the presence of G-F 20. This attenuating effect was not observed with non-elastoviscous hyluronan solutions of low MW.

Conclusion: Mechanosensory channels which are presumably involved in mechanotransduction have a decreased mechanical sensitivity in the presence of high MW hyluronan solutions. These results suggests that the analgesic effect of intra-articular injection of hylan elastoviscous solutions in animals and arthritic patients is due to a reduced responsiveness of knee joint pain receptors.

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M44

PAIN RELIEF IS NOT A CONFOUNDER IN JOINT SPACE NARROWING ASSESSMENT OF FULL EXTENSION KNEE RADIOGRAPHS

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Background: Presence of pain may impair knee full extension in weight-bearing antero-posterior radiographs using such patient positioning and view, in osteoarthritis (OA) disease modification studies.

Objectives: To assess whether improvement in knee pain improved the fully extended knee position, resulting in an artifactual increase in joint space width (JSW) in two recent trials showing symptom and joint structure modification with glucosamine sulfate (GS).

Methods: 212 and 202 patients with knee OA (ACR criteria), respectively (total 414), were randomised to double-blind treatment with oral GS 1500 mg u.i.d. or placebo for 3 years. JSW was assessed with the aid of a magnifying lens at the narrowest point of the medial compartment of the tibio-femoral joint, and symptoms were assessed by the WOMAC index. For the purpose of this analysis, 3-year completers were selected based on a cut-off of improvement in the WOMAC pain subscale equal to the mean improvement observed in the GS group in each study. The average improvement in WOMAC pain and the mean (ES) change in JSW were calculated for these patient subsets and compared between treatment groups.

Results: Data in the combined analysis reported here reflect the results in each study, that were similar. There were obviously more patients above the selected pain improvement threshold with GS (74 vs 56 with placebo, in the two studies combined), but the two subsets has a similar baseline level of mild to moderate pain and JSW around 4 mm, similarly to the overall treatment groups. Notwithstanding a major decrease in WOMAC pain of comparable size in both patient subsets, -59% with GS vs –51% with placebo

(NS), the placebo patients underwent a definite joint space narrowing (JSN) of –0.23 (0.11) mm that was not observed with GS, +0.14 (0.06) mm: p=0.005.

Conclusion: Major relief in mild to moderate pain is not a confounder in the evaluation of JSN on weight-bearing knee radiographs taken in full extension. The structure modifying effect of GS is not an artifact of the drug symptomatic activity.

M45

CARTILAGE DEFECTS, SUBCHONDRAL BONE MARROW CHANGES, PAIN AND KNEE OSTEOARTHRITIS

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Aims: Determine if the presence of subchondral bone marrow abnormalities (BME) and cartilage defects, would explain the difference between painful osteoarthritis of the knee (OAK) vs. painless OAK vs. pain without OAK.

Methods: Magnetic resonance imaging (MRI) with 1.5 T scanner of four groups of 120 women (30 per group), aged 35-55 years, were recruited from the Southeast Michigan Osteoarthritis cohort (1-painful OAK; 2-painless OAK; 3-knee pain without OAK; 4-neither OAK nor knee pain). OAK was defined by a Kellgren-Lawrence score of 2 or greater while pain was based on self-report.

Findings: BME lesions were identified in 56% of all knees. BME lesions were 4 times (95 CI=1.7,8.7) more likely to occur in the painless OAK group as compared to the group with pain but no OAK. BME lesions > 1 cm were more frequent (OR=5.0, 95 CI=1.4, 10.5) in the painful OAK group vs. all others. While the frequency of BME lesions was similar in the painless OAK and painful OAK groups, there were more lesions > 1 cm in the painful OAK group. One-third of knees with full-thickness cartilage defects and 47% of knees with cartilage defects involving bone had BME > 1 cm. Women with painful OA and full-thickness articular cartilage defects with adjacent subchondral bone defects were significantly more likely to have painful OAK than other groups.

Conclusion: BME associated with full-thickness cartilage defects presented consistently with knee pain and is useful in explaining the difference between painful OAK vs. painless OAK.

M46

IMMEDIATE EFFECTS OF ADHESIVE TAPE ON PAIN AND PHYSICAL IMPAIRMENTS IN INDIVIDUALS WITH KNEE OSTEOARTHRITIS

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AIM OF STUDY: To evaluate the effects of two knee taping techniques (therapeutic tape and neutral tape) on pain and selected parameters of physical function in individuals with symptomatic knee osteoarthritis (OA).

METHODS: Using a within-subjects study design, 12 women and 6 men (mean (SD) age 66.9 (6.5) years) with symptomatic knee OA (as defined by the American College of Rheumatology) participated. Each participant was assessed under three testing conditions in random order (determined by balanced Latin square design): untaped, whilst wearing therapeutic knee tape and whilst wearing neutral knee tape. Therapeutic tape aimed to realign the patella and unload the infrapatellar fat pad, whilst neutral tape aimed to provide cutaneous input only. Physical impairment measures included: self-selected fast walking speed on a level surface, Timed Up and Go test, knee joint position sense, isometric quadriceps strength, electromyographic onset of quadriceps activity during stair descent, step test and postural sway. Severity of pain experienced during each physical test was measured on a visual analog scale. Results were analysed statistically using either a one-way repeated measures analysis of variance or the Friedman test. Paired t-tests or the Wilcoxin Signed Ranks test were used to locate the source of significant differences, using a Bonferroni adjusted α of 0.017.

RESULTS: Therapeutic tape significantly reduced pain during level gait, stair descent, step test and postural sway assessment when compared to the neutral and untaped conditions ($p<0.017$). Tape application had little effect on the physical function parameters investigated, with the exception of the step test, which was significantly improved by therapeutic tape when compared to the neutral and untaped conditions ($p<0.001$).

CONCLUSIONS: Therapeutic knee tape is a simple, inexpensive self-management strategy that may be used in the conservative treatment of knee OA. Whilst effective in immediately reducing pain on activity, it does not appear to have a significant immediate impact on physical impairments associated with the disease.

M47

OMERACT-OARSI Initiative: OARSI set of responder criteria for osteoarthritis (OA) clinical trials revisited

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Background: The OARSI Standing Committee for Clinical Trials Response Criteria Initiative had developed 2 sets of responders criteria permitting to present the results of changes after treatment in 3 symptomatic domains, *i.e.*. pain, function and patient's global assessment, as a single variable for clinical trials (1). For each domain, a response was defined by both a relative and an absolute change and with different cut-offs with regard to the drug, the route of administration and the OA localization.

Objective: To propose a simplified set of responder criteria with similar cut-off. whatever the drug, the route or the OA localization.

Methods: *Data driven approach:* Data from clinical randomized blinded placebo controlled trials were used to evaluate the performances of the two formal scenarios with 2 different databases ("elaboration" *versus* "revisit") and those of the 4 proposed simplified scenarios within the "revisit" database. The placebo effect, active effect, treatment effect, and the sample arm size needed to obtain the sensitivity and the specificity observed were evaluated for each of the 6 scenarios. *Expert's opinion approach:* Results were presented to the OMERACT expert members who selected the most appropriate set of responder criteria.

Results: *Data driven approach:* 15 studies involving 8164 OA patients were enrolled in the "revisit" database. The variability observed in the revisit database with the different sunplified scenarios was similar to the one observed between the 2 databases (elaboration *versus* revisit) for the formal scenarios. The treatment effect and the sample arm size required were similar for each set of criteria. *Expert 's opinion approach:* According to the experts, these two previous performances are the most important of an optimal set of responder criteria. Their choice fell on the set of criteria considering both pain and function as evaluation domain and requiring an absolute change and a relative change from baseline to define a response, with similar cut-off whatever the drug, the route of administration or the OA localization evaluated.

Conclusion: This data driven and expert's opinion approach has permitted to propose an optimal simplified set of responder criteria for OA clinical trials.

(l)Dougados M et al.. Osteoarthntis and cartilage, 2000. 8: 395-403.

M48

THE OSTEOARTHRITIS INITIATIVE: *A PUBLIC-PRIVATE PARTNERSHIP*

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OA is a major cause of disability and loss of quality of life. There are few if any current disease modifying drugs for OA. Clinical Indicators of OA are weak at best and include: pain, disability, joint space narrowing. Clinical endpoint studies are long and expensive with currently existing methodologies. Validated biomarkers are the key to shortening this process and improving decision making for drug development. In order to develop such biomarkers, we need a better understanding of the disease: etiology, onset, and progression.

A population-based longitudinal study of osteoarthritis would address these research needs and supply opportunities of further research in OA. It would provide resources to help remove the barriers that currently exist to moving research forward in the area of clinical OA. What role can NIH play in facilitating research advances?

The idea for an Osteoarthritis Initiative was conceived as a means to create a research resource to aid in the identification and evaluation of biomarkers as candidates for surrogate endpoints for OA. The mechanism was the development of a prospective, natural history cohort to be followed for 4-5 years. Clinical and imaging data and biospecimens will be collected. This Initiative is a public- private partnership with intellectual input and planning carried out by representatives from private industry, academic centers, government agencies, and private foundations. The financial support of this initiative will be provided by private industry and the government through the NIH.

What will result from the OAI? The recruitment of a new cohort, enriched for "at risk" individuals. The establishment of a public database that will provide research resources for biomarker validation in the forms of biological specimens, imaging files and paired clinical data. This research resource will stimulate basic research on biomarkers for OA and in turn, facilitate drug development through the identification of biomarkers of disease onset and progression. The long term result of this Initiative will be the confirmation of biophysical/ radiological, biochemical, genetic, and phenotypic patterns associated with onset and progression of OA leading to streamlined clinical trials, and a more thorough understanding of the disease and its manifestations in at risk populations. Such advances will lead to increased development of disease modifying drugs and treatments and more efficient safety and efficacy assessments in clinical trials. Positive, interactive relationships between agencies involved.

M49

QUANTITATIVE MORPHOLOGICAL ASSESSMENT OF CARTILAGE IN OA BY MAGNETIC RESONANCE IMAGING (MRI)

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Analyses of form-function relationships and disease processes in human articular cartilage required in vivo assessment of cartilage morphology and deformational behavior. MR imaging and advanced digital post-processing techniques have opened novel possibilities for quantitative analysis of cartilage morphology, structure, and function in health and disease. The presentation will review work on three-dimensional post-processing of MR image data of cartilage and summarize studies on their accuracy and precision in human joints. Particular focus will be on the validation of measurements in various stages of OA. We will present normative values on cartilage volume, thickness, and joint surface areas in the healthy human knee, and describe the correlation between different joint surfaces and that with gender, body dimensions, and age. We will summarizes ongoing work on functional adaptation of articular cartilage to mechanical loading, analyses of in situ cartilage deformation in intact joints in vivo, and the quantitative evaluation of cartilage tissue loss in osteoarthritis.

Specifically, we will describe dedicated algorithms for image segmentation, reconstruction, and image analysis (thickness, surface size, surface curvature). We will show that fat-suppressed gradient echo sequences permit highly accurate analysis of cartilage morphology, both in healthy and severely osteoarthritic joints. Reproducible measurements (CV% = 1 to 3 %) can be obtained in most compartments of the human knee, and also in joints with thinner cartilage (CV% = 2 to 10 % in the ankle). However, we will show that the precision is dependent on the specific section orientation and resolution. Data on the long-term vs. short-term precision of cartilage volume and thickness measurements will be presented as well as matching algorithms for analysis of regional/local changes in cartilage thickness. It will be shown that relatively small differences in cartilage morphology exist between both limbs of the same person (~5%), but large differences between individuals (CV% ~20%). Men display only slightly thicker cartilage then women (~10%), but significantly larger joint surface areas (~25%), even when accounting for differences in body weight and height. Weight and height represent relatively poor predictors of cartilage volume and thickness, but the size of the bone cartilage interface represents a useful parameter for normalizing cartilage volume measurements to achieve reasonable T-scores of cartilage loss in cross-sectional studies. The level of physical exercise does not appear to account for inter-subject differences in cartilage thickness. The thickness decreases slightly in the elderly, even in the absence of osteoarthritic cartilage lesions. Moreover, the deformational behavior of the cartilage decreases with age versus that in young healthy volunteers.

Application of these techniques to larger cohorts of patients in epidemiological and clinical studies will further establish the role of quantitative cartilage imaging in clinical research and in the management of OA. In particular we will describe how MR-based techniques can be potentially applied to evaluate SMOADs.

M50

NEW PERSPECTIVES IN THE MEDICAL MANAGEMENT OF OSTEOARTHRITIS

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Until recently, the main aim of pharmacological treatments of osteoarthritis has been to control the symptoms of the disease. On the other hand, due to their mechanism of action, specific compounds may not only modify the course of symptoms over long-term treatment periods, but also favourably affect joint structure changes and thus the disease progression.

The structure-modifying properties of Chondroitin sulfate (CS) were assessed in a double-blind, placebo-controlled trial that included 119 patients with interphalangeal OA. After 3 years, the group taking 400 mg CS three times daily had significantly fewer patients with new erosive OA finger joints (8,8 %) compared to the placebo group (29,4 %). More recently, a dose of 800 mg/day of chondroitin sulfate, given for a period of 2 years to patients with primary knee osteoarthritis, prevented the decrease in joint space width and joint space thickness observed in patients having received the placebo for the same duration. To test the long-term effects of Glucosamine sulfate (GS) on the progression of OA joint structural changes in symptoms of the knee 212 patients with knee OA were randomly assigned in a double-blind fashion to a continuous treatment with GS (1500 mg once/day) or placebo for 3 years. Patients treated with placebo had an average joint space narrowing (JSN) of approximatively 0.08 to 0.1 mm/year, while no joint space narrowing occured in the group treated with glucosamine sulfate. These results were later confirmed by another double-blind, placebo-controlled study, performed in 202 patients with knee OA. In the glucosamine group, the minimum joint space width of the tibio-femoral joint increased by 0.02 mm after 3 years while it decreases by 0.19 mm in the placebo group. In a three-year, randomized, double-blind, placebo-controlled study, 507 patients with severe to moderate hip osteoarthritis were randomized to receive either diacerein, 50 mg twice daily or identical placebo. At the end of the trial, 62.7 % of the placebo and 53.7 % of the diacerein treated patients ($p = 0.037$) had a radiological progression defined as a reduction in the joint space width of at least 0.5 mm. Within the valid completers population (n=269), the annual joint space narrowing rate was significantly lower in those receiving placebo (0.018 mm/year versus 0.023 mm/year; $p=0.042$).

There is now a convergent body of evidence that several substances acting either through a synthesis of matrix constituants or through an inhibition of catabolic mediators and processus can significantly interact with the progression of osteoarthritis. The most conclusive evidence stands with glucosamine sulfate.

M51

ISOMERIZED AGGRECAN FRAGMENTS ARE RELEASED DURING CARTILAGE DEGRADATION AND CAN BE MEASURED IN SERUM

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Isomerization of asparaginyl and aspartyl residues is a posttranslational process occurring spontaneously at susceptible sites in long-lived proteins. In tissues with low turnover rate such as cartilage, significant amounts of age-modified proteins may accumulate.

Our aim was to characterize isomerization prone aspartyl/asparaginyl residues (iso-D) in non-collagenous cartilage proteins.

A human cartilage sample obtained from a recently deceased human male donor (age 60 years) was extracted with 4 M Guanidinium-HCl and subsequently centrifuged at 27,000 x g. Aliquots of the supernatant were subsequently reduced and digested with trypsin. Tryptic digests were subjected to size-exclusion chromatography (SEC) and further purification using reverse phase HPLC. Eluents were collected in fractions and measured in an enzyme-assay employing the enzyme L-Iso-Aspartate Methyl Transferase (IAMT, EC 2.1.1.77) for detection of iso-D containing protein fragments. Fractions containing iso-D sites as identified with the IAMT enzyme were purified to homogeneity and subsequently subjected to amino acid sequencing and mass spectrometry to identify the cartilage protein fragments containing iso-D modifications.

Iso-D sites were identified in aggrecan as well as other cartilage matrix proteins. Synthetic peptides representing several of these sites were synthesized and used to generate immunoassays, for measurement of iso-D containing aggrecan fragments in circulation. Fragments of aggrecan containing an isomerized form of the epitope GRVRV-isoD-SAY were found in circulation. The levels of such fragments were elevated in OA and RA patients suggesting that they may provide a novel biochemical marker of cartilage catabolism.

Further studies are needed to assess the functional significance of these spontaneous posttranslational modifications. The clinical potential of isomerized aggrecan fragments to reflect cartilage degradation in arthritis and other conditions involving elevated joint tissue metabolism is currently being investigated.