612 EARLY SUBCHONDRAL BONE CHANGES IN AN OSTEOARTHRITIS MODEL IN WILD TYPE AND TGF-ALPHA KNOCKOUT MICE

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Purpose: To examine early subchondral bone changes in the DMM (destabilization of medial meniscus) model of injury-induced osteoarthritis (OA) in wild type (Wt) as well as in TG Falpah knockout (KO) mice.

Methods: C57/Bl6 Wt and TG Falpah KO mice were subjected to DMM or SHAM surgery at 12-weeks of age and harvested at 2-, 5- and 10-weeks post-surgery. Catwalk gait analyses, Micro-Computed Tomography (µCT), Toluidine Blue (TB), Picrosirius Red (PR) and Tartrate-Resistant Acidic Phosphatase (TRAP) staining of paraffin-embedded sections were used to investigate gait patterns, 3D joint morphology, bone mineral density (BMD) of subchondral bone, histology, collagen organization and activity of osteoclasts.

Results: In Wt mice, OA severity progressed from mild or moderate (5-week post-surgery) to severe (10-week post-surgery) according to OARSI histopathology scoring. Gait disparity occurred only at 10-week post-surgery in DMM mice but not at earlier time-points. Osteophyte formation around the surgical joint was noticed as early as 2-week post-surgery in DMM mice but not at earlier time-points. Osteophyte formation around the surgical joint was noticed as early as 2-week post-surgery in DMM mice but not at earlier time-points. Osteoclast activity increased during OA and reverted to baseline at later stages. Gait disparity in mice only occurred at 10-week post-surgery. Catwalk gait analyses, Micro-Computed Tomography (µCT), Toluidine Blue (TB), Picrosirius Red (PR) and Tartrate-Resistant Acidic Phosphatase (TRAP) staining of paraffin-embedded sections were used to investigate gait patterns, 3D joint morphology, bone mineral density (BMD) of subchondral bone, histology, collagen organization and activity of osteoclasts.

Conclusions: BMD of subchondral bone increased at the early stages of OA and reverts to baseline at latter stages. Gait disparity in mice only occurred at later stages of OA. Osteoclast activity increased during OA progression. TG Falpah KO might be protective for articular cartilage but detrimental for bone.

613 CORRELATION OF SUBCHONDRAL BONE MORPHOMETRY AND OARSI GRADE IN OSTEOARTHRITIC HUMAN KNEE SAMPLES

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Purpose: Osteoarthritis (OA) is a disease of a whole joint and there is increasing evidence that the subchondral bone contributes or coincides significantly to the pathogenesis of OA. Yet most of the diagnostic methods are focused mainly on cartilage degeneration and erosion. Consequently, there is a lack of data about relationship between bone morphometric and densitometric parameters of human trabecular bone and subchondral plate in relation to the actual histological degeneration of the overlaying cartilage. The aim of this study was to characterize morphological changes in human tibial trabecular bone and subchondral plate as a function of histological progression of OA.

Methods: 25 osteochondral samples were prepared from 13 OA patients treated with total knee arthroplasty at Oulu University Hospital. Samples were prepared from tibial plateaus which are always extracted during total knee replacement surgery. Tibial plateaus were visually classified into three categories in terms of degeneration of the articular cartilage: 1) most inviolable (or intact) cartilage, 2) moderate cartilage degeneration, and 3) partly or fully exposed subchondral bone. Cylindrical osteochondral samples with a diameter of 6 mm were prepared from all macroscopic visual grades. Samples were stored in phosphorus-buffered saline (PBS) for µCT imaging. Osteochondral cylinders were scanned with µCT device at isotropic 27.8 µm voxel size (SkyScan 1172, Bruker microCT, Kontich, Belgium). Images were reconstructed and analyzed with software package provided by the manufacturer. Finally a novel 3D local binary pattern (LPB) analysis was performed for volumetric dataset with a custom made Matlab program. After the µCT imaging, cylinders were formalin-fixed, paraffin embedded and sectioned. Sections of 5 µm were stained with Safranin-O. Histological sections were graded by three independent evaluators according to the standardized OARSI grading system. Final OARSI grade was defined as an average from three evaluators, which was then correlated with bone architectural and densitometric properties (Figures 1 and 2).

Results: All knees had large internal variation in OARSI grades depending on the site. Most of the analyzed morphological parameters showed significant increase with increasing OARSI grade in both trabecular bone compartment as well as in subchondral bone plate. Trabecular bone volume fraction, number and thickness increased 27%, 123% and 54%, respectively, between OARSI grades 1 and 6. At the same time trabecular separation and structure model index decreased by 46% and 70%, respectively. Subchondral plate thickness increased from 250µm to 810µm and bone specific surface decreased from 8.1% to 3.1%, between OARSI grades 1 and 6. Furthermore, local binary pattern analysis showed that there was a significant increase of different local patterns in trabecular bone, whereas in subchondral bone plate there was a significant decrease of different local binary patterns when normalized to number of analyzed voxels. Interestingly, we did not observe any changes in tissue mineral density (excluding pores) or porosity of subchondral bone plate when OARSI grade was increased.

Conclusions: This study further highlights the importance of subchondral bone changes in OA and demonstrates feasibility of using tissue material which is discarded during endoprosthetisation and which appears to contain representative variety of samples with different OA grades. Increased sclerosis of subchondral bone had strong association with the OARSI grade similarly to the trabecular volume fraction. This was not only due to an increase in trabecular number but the trabeculae became also thicker and more closely packed leading to more plate-like shape. The LPB analysis of trabecular bone indicated more complex patterns for higher OARSI grades. Conventional morphometric analysis of subchondral bone plate showed significant thickening of subchondral bone plate, while reduced bone specific surface with constant porosity indicates of reduced surface roughness that could be also detected in 3D models (figure 1). Similar conclusion was also supported by LPB analysis. This study highlights the features of bone architecture changes in the progression of OA.
Figure 2. Correlations between OARSI grade and properties of subchondral plate (grey dots) and trabecular bone (orange dots). On the left column, A) subchondral plate thickness (mm), C) specific bone surface (mm$^{-1}$) and E) number of different local binary patterns. On the right column, B) trabecular bone volume fraction and D) trabecular number (mm$^{-1}$) and F) number of different local binary patterns. ***p<0.001.

614 EFFECT OF CHONDROITIN SULPHATE AND GLUCOSAMINE IN COMBINATION IN AN ANIMAL MODEL OF OSTEOARTHRITIS AND OSTEOPOROSIS

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Purpose: Osteoarthritis (OA), the most common form of arthritis, is now recognized as a disease of the whole joint involving all joint tissues. Cartilage loss is a major pathologic feature of OA, but in addition it is suggested that subchondral bone might play an important role in the pathogenesis of OA. In the present study we explored whether the combination of chondroitin sulphate (CS) and glucosamine hydrochloride (GLU), both symptomatic slow-acting drug for osteoarthritis (SYSDOA), could protect subchondral bone and articular cartilage from degeneration in a combined rat model of Osteoporosis (OP) induced by ovariectomy and OA induced by anterior cruciate ligament transection (ACLT).

Methods: OP was induced by ovariectomy in female Wistar rats (180-200 g body weight, n = 15 rats/group)(week 0) and 2 weeks after (week 2) OA was induced by Anterior Cruciate Ligament Transection (ACLT). All surgical procedures were carried out under deep anaesthesia with isoflurane (1.5 minimum alveolar concentration) which was followed by the subcutaneous injection of butorphanol (2mg/kg). Animals were maintained at a temperature of 21±2°C with a 12 hour light/dark cycle and with free access to food and tap water. The combination was administered daily (oral gavage) at two doses from week 0 until week 12 after ovariectomy. The low dose was 140 mg/kg/day and the high dose 175 mg/kg/day which correspond approximately to 1200 mg/day and 1500 mg/day in humans. A Control Group and an ovariectomized + ACLT Group (Vehicle Group) were also included. After week 12, animals were sacrificed. For the assessment of OA, histology was performed and cartilage degeneration was evaluated by means of the OARSI score. Bone microarchitecture was assessed by microCT.

Results: Treatment has been shown to induce a significant reduction (approximately 80%) of the cartilage degradation and of the proteoglycan depletion (approximately 70%). This was accompanied by a significant reduction of Metalloprotease-3 and IL-1 levels. In addition, microCT revealed that the treatment exerted a positive effect in bone structure. This protective effect in bone loss was correlated with an increased ratio of Osteoprotegerin/RANKL.

Conclusions: The combination has been shown to induce significant effects in osteoarthritis and to have a protective role in bone. All these data may help to understand how Chondroitin and Glucosamine exert a positive effect in Osteoarthritis pathophysiology.

615 A COMPARATIVE STUDY OF DIAGNOSTIC AND IMAGING TECHNIQUES OF THE TRAPEZIUM BONE IN RIZOAORTHRISIS

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Purpose: Osteoarthritis of the trapeziometacarpal joint, known as rizoarthrosis, is a common rheumatic and disabling condition that occurs in 20% of women and 6% of men over 45 years old. At present, conventional radiography is the gold standard diagnostic tool for the clinical evaluation of the severity and progression of rizoarthrosis. Although x-ray is a fast, inexpensive, non-invasive and non-destructive technique, its inability to perceive precisely the articular cartilage and other structures offers a scarce diagnosis and incomplete details on subchondral bone defects. The aim of our research is to determine whether micro-CT can be regarded as a reliable investigation method to evaluate the severity of osteoarthritis in the trapezium bone, in particular, we developed a novel micro-CT scoring system based on a quantitative assessment of the subchondral bone thickness.

Methods: This study compared different diagnostic and imaging techniques performed consecutively on each sample: x-ray, visual analysis,