**Methods:** Expression of RORalpha isoforms was analyzed by semi-quantitative reverse transcriptase chain reaction (RT-PCR) and immunocytochemistry. RORalpha overexpression and down-expression were achieved by the transfections of expression vector and small interfering RNA (siRNA) of RORalpha1, respectively. Their effects on the modulation of osteoblast metabolism markers and tumor necrosis factor alpha (TNFalpha) induced inflammatory response markers were determined by real-time RT-PCR, western blot, enzyme-linked immunosorbent assay, gene reporter luciferase assay and electrophoretic mobility shift assay.

**Results:** The overexpression of RORalpha1 increased alkaline phosphatase (ALP), osteocalcin (OC) and collagen type I (COL I) mRNA and activity or protein expression, while the silencing of RORalpha1 RNA inhibited these responses. In addition, overexpression of RORalpha1 suppressed TNFalpha-induced production of cyclooxygenase-2 (COX-2), prostaglandins E2 (PGE2) and metalloproteinase-9 (MMP-9). Upon examination of signaling pathways, we found that RORalpha1 was able to block TNF alpha-induced nuclear factor kappa B (NF-kB) activation.

**Conclusions:** RORalpha1 is involved in human osteoblast metabolism by stimulating osteoblast marker expression and inhibiting inflammatory responses. These findings may encourage further exploration of RORalpha1 as a potential target for the treatment of bone disorders related to inflammation.

**OSTEOCLAST PHENOTYPE AND RESORPTIVE ACTIVITY ARE MODIFIED DISTINCTLY BY DIFFERENT TYPES OF OSTEOCLAST INHIBITORS - IMPLICATIONS FOR OSTEOCLAST QUALITY?**

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**Purpose:** Osteoarthritis (OA) is described as being a disorder that has its origin in bone and cartilage. It is therefore interesting to investigate the biological action of bone resorbing cells also known as the osteoclasts. Osteoclast inhibition can be used to investigate the biological processes underlying bone remodeling; however the direct effect on osteoclast phenotype caused by modulation of both the organic and inorganic phase of bone during resorption has not been investigated in detail. The aim of the current study was to investigate the phenotype of resorbative human osteoclasts after treatment with different types of inhibitors and thereby gain more knowledge about bone turnover.

**Methods:** Mature human osteoclasts, generated from CD14+ monocytes, were seeded on bone slices and treated with inhibitors of acidification (bafilomycin; diphlin; ethoxyzolamide), inhibitors of proteolysis [E64 [cat. K inhibitor]; GM6001 [MMP inhibitor]] or a bisphosphonate (Ibandronate). Bone resorption was measured by Ca2+ (inorganic), CTX-I (organic), ICTP (organic, MMP generated) and pit scoring. In addition, gelatinase activity and the osteoclast marker TRACP were measured.

**Results:** All inhibitors of acidification were equally potent with respect to inhibition of organic and inorganic resorption, measured both by resorption markers and pit scoring. Conversely, E64 ef- fectively reduced organic resorption by 80%, whereas inorganic resorption was modestly reduced. Resorbed bone area, ICTP release and gelatinase activity were all increased for this treat- ment. Treatment with GM6001 had no effect on neither organic nor inorganic resorption alone; however, when combined with E64 degradation of the organic phase of bone was abrogated, whereas inorganic resorption was reduced by 60%. Ibandronate completely abrogated both organic and inorganic resorption, while TRACP activity was strongly decreased.

**Conclusions:** Inhibitors of acidification and ibandronate potently reduced both organic and inorganic resorption to the same level. In face of that, inhibition of proteolysis leads to potent reduction of organic resorption, but only modest reduction of inorganic resorption, and even an increased resorbed bone area, possibly due to MMP mediated compensation. These findings strongly indicate that different anti-osteoclastic intervention strategies affect...
osteoclasts distinctly, resulting in separate osteoclast phenotypes. Since bone formation is coupled to bone resorption, the multi-layered osteoclast phenotype and quality may be related to bone turnover and bone quality, which could prove important for the pathogenesis of OA.

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HYALURONAN AND INTERMITTENT HYDROSTATIC PRESSURE SYNERGISTICALLY SUPPRESSED MMP-13 AND IL-6 EXPRESSIONS IN OSTEOBLASTS FROM OA SUBCHONDRAL BONE

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Purpose: Various inflammatory cytokines and proteases are involved in the initiation and progression of osteoarthritis (OA). We previously reported that the expressions of matrix metalloproteinase-13 (MMP-13) and interleukin-6 (IL-6) were augmented in OA subchondral bone. The purpose of this study was to investigate the effects of hyaluronic (HA) and mechanical stress on osteoblasts isolated from OA subchondral bone.

Methods: OA subchondral bone from the distal end of the femur was harvested from 9 patients at total knee arthroplasty. The subchondral bone underlying the degenerated articular cartilage was cut into small pieces and incubated in DMEM for 3 weeks, and then osteoblasts were isolated.

Subchondral bone osteoblasts (SBOs) were cultured with DMEM containing 30% fluorescent labeled HA for 48 hours and washed twice with PBS. The monolayer cultured cells were observed with a fluorescence microscope. As control, SBOs cultured without fluorescent labeled HA was used. SBOs were divided into 4 experimental groups. Control group: cultured without stimulation, HA group: incubated with HA (1000 mg/ml, 48 hours), IHP group: applied intermittent hydrostatic pressure (IHP) (1/2 Hz, 5 MPa, 60 minutes), and HA+IHP group: incubated with HA followed by IHP. Total RNA were extracted and mRNA expression was examined by real-time RT-PCR for MMP-13 and IL-6. In control group and HA+IHP group, culture supernatant was harvested 24 hours after the application of HA + IHP, and concentrations of IL-6 and MMP-13 were measured using an enzyme-linked immunosorbent assay (ELISA). Values were analyzed statistically by Tukey-kramer’s test and paired t-test and a p value less than 0.05 was considered significant.

Results: In the fluorescent labeled HA group, fluorescence was observed in the area of cytoplasm but not in nuclei 48 hours after the administration. The mRNA expressions of MMP-13 of the each group compared to the control group were 101±18.2%, 89.3±14.1% and 51.2±7.5% respectively, indicating that MMP-13 expression in the HA + IHP group significantly decreased compared to those in the control group and in the HA group.

The IL-6 mRNA of each group was 76.6±11.9 %, 73.2±10.5% and 54.0±18.3%, indicating that IHP treatment and HA + IHP treatment significantly suppressed the IL-6 mRNA. The production of MMP-13 and IL-6 were 51.3±16.6 (pg/ml) and 70.8±25.6 in the control group. In the HA + IHP group, they were significantly reduced to 53.9±11.7 and 45.2±11.1.

Conclusions: The role of subchondral bone attracts attention in the onset and/or progression of OA. It was reported that HA influences metabolism in subchondral bone, and that subchondral bone becomes more compliant and thereby reduces cartilage stress. However, the mechanism of the influence of HA on subchondral bone remains unclear. In this study, HA was exposed to osteoblasts, and the enhanced expressions of MMP-13 and IL-6 in OA osteoblasts were significantly suppressed by HA in combination with IHP. In the natural course of OA, a resorption of subchondral bone was documented in the early stage of OA and was considered to take an important role in the progression of the disease. In OA, neovascularization between cartilage and subchondral bone is observed while tidemark disappears. Therefore, intra-articular injection of HA in combination with appropriate exercise could suppress MMP-13 and IL-6 expressions in subchondral bone, which may prevent abnormal metabolism in osseous tissue in OA.

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INCREASE IN THE MEDIAL CORTICAL BONE THICKNESS OF THE PROXIMAL TIBIA IS ASSOCIATED WITH THE PROGRESSION OF RADIOGRAPHIC KNEE OSTEOARTHRITIS (OA) IN RURAL JAPANESE POPULATION - THE MATSUDAI KNEE OA SURVEY

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Purpose: While increased subchondral bone thickness has been well described in knee OA, changes in periarticular cortical bone in OA patients have not been previously examined. The purpose of this study is to investigate the association of cortical bone thickness at proximal tibia in knee OA with varus deformity.

Methods: The Matsudai Knee OA Survey is a historical cohort study, which was conducted to investigate the risk factors of the disease in a rural Japanese population. We have conducted an extensive the survey in Matsudai town Niigata city, Japan since 1979. Matsudai knee OA survey was performed every 7 years. In this survey, check-up included an interview, physical examination, anteroposterior standing radiographs of both knees. OA was defined as being present in a knee if radiographic grade of 2 or higher with Kellgren and Lawrence (KL) scale were detected. Mal-alignment was determined according to the femoro-tibial angle (FTA), and subjects were grouped into higher and lower FTA by median FTA. In this study, we describe the cross-sectional data collected from the 4th (2000) survey, which included 1260 people (396 men and 527 women). Both medial and lateral cortical bone thicknesses at proximal tibiae and femora were measured using computer image analysis. Cortical bone thickness was compared with KL grades, FTA groups (higher and lower groups divided median FTA), BMI (4 groups of quartile).

Results: In female, both medial and lateral cortical bone thickness of femur and lateral side of tibia decreased with age, but the medial cortical thickness was not decrease with age. In male, only lateral cortical thickness was decrease with age. Cortical bone thickness,