**Methods:** Expression of RORalpha isoforms was analyzed by semi-quantitative reverse transcriptase chain reaction (RT-PCR) and immunocytochemistry. RORalpha1 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 TNFα-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.

162

A LOWER OSTEOPROTEGERIN/RANKL RATIO IS ASSOCIATED WITH AN INCREASED SUBCHONDRAL BONE REMODELING IN OSTEOARTHRITIS AGGRAVATED BY PRIOR OSTEOPOROSIS IN RABBITS

M. Bellido1, L. Lugo1, S. Castañeda2, R. Largo1, G. Herrero-Beaumont1

1 Fundacion Jimenez Diaz, Madrid, Spain; 2 Hosp. de la Princesa, Madrid, Spain

**Purpose:** In previous studies, we have observed that prior subchondral bone (SB) osteoporosis (OP) aggravated the cartilage damage in a combined model of OP and osteoarthritis (OA) in rabbits (Calvo et al. Osteoarthritis Cartilage 15:69-77, 2007). In this work, our aim was to study whether an increased SB remodeling could account for the increased cartilage damage in rabbits with OA aggravated by prior subchondral OP.

**Methods:** OP was induced in 20 (8 month-old; 3.5-4.8 kg body weight), skeletally mature female NZ rabbits, by ovariectomy (OVX) and intramuscular injections of methylprednisolone hemisuccinate (1 mg/kg/day for 4 weeks; OP group). Ten age and gender-matched additional animals were used as controls. Surgical OA was simultaneously induced in the left knees of all the rabbits through partial medial meniscectomy and anterior cruciate ligament transection. The animals were sacrificed 22 weeks after OVX. Then, left knees were considered as OA or as OA plus OP (OPOA) and the right knees were used as OP or healthy controls, respectively. The percentage of bone area/tissue area (BAr/TAr) was assessed in the SB of the femurs after sacri- fice by micro-Computed Tomography. Osteoprotegerin (OPG), RANKL and MMP-9 protein synthesis at the SB were evaluated both analyzed in the SB, were diminished in OA, OP and even more in OPOA. In the same way, the MMP-9 synthesis was greater in OPOA than in OA rabbits. Our results show that an increased SB remodeling can be responsible for the severity of cartilage damage in OA, at least in rabbits with prior OP. This finding can be relevant since these pathologies are frequently found in the same patients, in which OP can precede OA onset.

163

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

A.V. Neutzsky-Wulff, M.G. Soerensen, D. Kocijancic, A.-C. Bay-Jensen, M.A. Karsdal, K. Henriksen

**Nordic BioSci. A/S, Herlev, Denmark**

**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 resorptive 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; diphosphonate; 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 increased.

**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...