that CM is able to increase subchondral bone mineral density (BMD) in an experimental model of osteoporosis.

Objectives: To evaluate if CM could prevent the subchondral BMD alterations induced by OA, in association to an improvement in synovial membrane inflammation and cartilage damage in an OA model in rabbits.

Methods: Ten male New Zealand rabbits were submitted knee surgery to induce OA by transection of anterior cruciate ligament. CM was performed using the chiropractic adjusting instrument Activator V 3 times a week during 8 weeks as follows: Force 2 setting was applied onto the tibial tubercle of the rabbit right hind limb (CM-OA group), at an angle of 90°; whereas the corresponding left hind limb received a false manipulation (FM-OA group) consisting of Activator V firing in the air and touching the tibial tubercle. Three healthy animals were used as controls. Following sacrifice, tibiae and femora were removed for mCT and histological evaluation.

Results: In the OA rabbits, subchondral BMD decreased in relation to control animals (OA 4.72±0.19 vs Control 5.18±0.2 mg/cc), been partially reversed in the tibia of OA rabbits with CM (TM-OA 5.05±0.216 vs FM-OA 4.40±0.170 mg/cc). Subchondral trabecular bone structural parameters were analysed by microCT and a significant decrease of bone volume/trabecular volume (BV/TV), trabecular number (TbN) and trabecular thickness (TbTh) was observed in the OA rabbits, while trabecular separation (TbS) increased compared to control animals. TM-OA group showed a significant improvement of these parameters compared to FM-OA group. TM-OA had lower cartilage damage compared to FM-OA (TM-OA 4±0.67 AU vs FM-OA 8±1.25 AU). TM-OA synovial membranes presented a total Krenn score lower than FM-OA joints (FM-OA 2±0.35 vs FM-OA 4.5±0.38 AU). OA synovial membranes showed higher levels of Collagen VI respect to control ones; TM-OA synovial membranes presented less expression of Collagen VI than FM-OA group (TM-OA 1.4±0.13 vs FM-OA 2.2±0.3 AU), been this associated with a decrease of both MMP3 (TM-OA 1.2±0.1 vs FM-OA 1.7±0.2 AU) and VEGF (TM-OA 1.2±0.14 vs FM-OA 1.9±0.26 AU).

Conclusions: These results support the hypothesis that CM may ameliorate subchondral BMD alterations induced by OA, in association to an improvement on synovioathy and cartilage degradation.

REFERENCE:

Disclosure of Interest: None declared.


AB0096 EXPRESSION AND FUNCTION OF NEUROPEPTIDE Y RECEPTORS IN HUMAN ARTICULAR CARTILAGE: INFLUENCE OF GENDER AND OSTEARTHRITIS

H. Yazawa, M. Yanagisawa, Y. Azaki, Y. Araki, K. Sato, T. Mimura. Department of Rheumatology and Applied Immunology, Saitama Medical University, Saitama, Japan

Background: In rheumatoid arthritis (RA), inflammatory synovial tissue called the pannus proliferates and erodes the articular cartilage and bone in the affected joints. Osteoclasts, multinucleated cells of monocyte/macrophage lineage, are involved in the bone destruction in RA. Thus, osteoclasts are considered an important therapeutic target in the prevention of the joint destruction. Mouse bone marrow cells differentiate into osteoclasts when co-cultured with osteoblasts or stromal cells for osteoclastogenesis in place of osteoblasts. Synovial fibroblasts cannot be substituted for osteoblasts in a co-culture system of osteoclast differentiation. This is probably because synovial fibroblasts do not provide sufficient RANKL and M-CSF. Instead, they produce a large amount of OPG. This may be a mechanism by which ectopic osteoclastogenesis is inhibited. Thus, the osteoclasts observed in the pannus may be dependent on membrane-bound RANKL from other sources, like lymphocytes, or may be differentiated by stimulation with cytokines other than RANKL, such as TNF-α and IL-6.

Methods: Synovial tissues were obtained from RA patients who underwent joint replacement surgery. Mouse osteoblasts were obtained from the calvariae of 2- or 3-day-old newborn C57BL/6 (B6) mice. Mouse bone marrow cells were prepared from femoral bones. Osteoclasts were visualised with tartrate-resistant acid phosphatase (TRAP) staining. The protein levels of RANKL and its decoy receptor, osteoprotegerin (OPG), in the culture supernatant were quantified using ELISA.

Conclusions: Synovial fibroblasts cannot be substituted for osteoblasts in a co-culture system of osteoclast differentiation. This is probably because synovial fibroblasts do not provide sufficient RANKL and M-CSF. Instead, they produce a large amount of OPG. This may be a mechanism by which ectopic osteoclastogenesis is inhibited. Thus, the osteoclasts observed in the pannus may be dependent on membrane-bound RANKL from other sources, like lymphocytes, or may be differentiated by stimulation with cytokines other than RANKL, such as TNP-α and IL-6.

REFERENCES:

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AB0097

INDIVIDUAL FUNCTIONS OF THE HISTONE-ACETYLATION MODIFIERS CBP AND P300 IN RHEUMATOID ARTHRITIS SYNOVIAL FIBROBLASTS

G. Lee1, C. Kolling1, O. Distler2, C. Ospekt1, K. Klein3, 1Department of Rheumatology, Center of Experimental Rheumatology, University Hospital Zurich, Schlieren, 2Schulthess Clinic, Zurich, Switzerland

Background: The close homologues cAMP-response element binding protein (CREB) binding protein (CBP) and p300 are writers of H3K27 histone acetylation marks found in active enhancers. In addition, their bromodomains are readers of acetylated lysine residues on histone tails and are subject of drug development efforts. CBP and p300 are widely accepted as transcriptional coactivators which play important roles in regulating energy metabolism, survival, apoptosis resistance, adherence and invasiveness of adjacent tissues. Periarticular tumor formation in rheumatoid arthritis (RA) manifests tumor-like properties including increased proliferation, prolonged survival, adipose resistance, adherence and invasiveness of adjacent tissues. Periarticular tumor formation in RA has been suggested to involve increased expression of inflammatory and matrix-degrading targets.

Objectives: To investigate the roles of PGC-1β on regulating migration and invasion of RA-FLS in RA.

Methods: RA-FLS were treated with the pan inhibitor I-CBP (1 μM, 5 μM), targeting the bromodomains of CBP and p300, in presence and absence of TNF-α (10 ng/ml) for 24 hour. The expression of CBP and p300 was silenced by transfection of antisense LNA gapmer (5 nM) in SF. Knockdown was verified by Western blotting. 24 hour after transfection cells were stimulated with TNF-α (10 ng/ml) for 24 hour. The mRNA expression of potential target genes was measured by quantitative Real-time PCR, using RPLP0 as an endogenous control.

Results: I-CBP dose-dependently reduced the TNF-α-induced expression of MMP1 (p<0.05), MMP3, IL6 and IL8 in SF (n=3). Antisense LNA gapmer targeting CBP reduced the protein expression of CBP by 68.7% (±12.9%, p<0.01, n=5) in unstimulated cells and by 89.7% (+12.9%) in presence of TNF-α. The protein expression of p300 was reduced by 55% (±29.8%, p<0.05, n=6) in unstimulated cells and by 62.7% (+27.9%) in presence of TNF-α after transfection of LNA gapmers targeting p300. Silencing of CBP in SF (n=7) reduced the TNF-α-induced expression of IL6 (p<0.05), IL8 (p<0.05), MMP3 (p<0.05), as well as the basal (p<0.01) and the TNF-α-induced expression of MMP1 (p<0.05). In contrast, silencing of p300 induced the basal expression of IL6 (p<0.01), MMP1 (>0.05), and MMP3 (p>0.05), as well as the TNF-α-induced expression of IL6 (p<0.078), IL8 (p<0.078), MMP1 (p>0.05) and MMP3 (p<0.063). Silencing of CBP in hand SF (n=4) reduced the expression of hand-specific HOX genes including HOXD10 (0.53±0.10 fold; p<0.01), HOXD11 (0.75±0.19 fold; p<0.098) and HOXA13 (0.75±0.17 fold; p=0.063), whereas HOX4A, HOXAl0 and HOXAl1 were not affected. Silencing of p300 reduced the expression of HOXD10 (0.65±0.24 fold; p<0.061), HOXD11 (0.45±0.10 fold; p<0.010), HOXA10 (0.70 ±0.14 fold; p<0.005) and HOXA13 (0.55±0.19 fold; p<0.05). The down regulation of HOXD10 after silencing of CBP and p300 in hand SF was confirmed on protein levels by Western blotting.

Conclusions: Our results unravel opposing functions of CBP and p300 in regulating the TNF-α-induced expression of inflammatory and matrix-degrading target genes in SF. In addition, CBP and p300 likely contribute to the maintenance of a joint-specific gene expression in SF by regulating the expression of hand-specific HOX genes.

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