Objectives: we investigated that BA could suppress RANKL-induced osteoclastogenesis and bone resorption.

Results: BA significantly suppressed osteoclastogenesis by decreasing the phosphorylation of Akt and I κ B, as well as PLC γ 2-Ca²⁺ signaling, in pathways involved in early osteoclastogenesis as well as through the subsequent suppression of c-Fos and NFATc1. The inhibition of these pathways by BA was once more confirmed by retrovirus infection of constitutively active (CA)-Akt and CA-I κ B β retrovirus and measurement of Ca²⁺ influx. BA also significantly inhibited the expression of osteoclastogenesis-specific marker genes. Moreover, we found that BA administration restored the bone loss induced through acute lipopolysaccharide injection in mice by a micro-CT and histological analysis.

Conclusion: Our findings suggest that BA is a potential therapeutic candidate for bone diseases involving osteoclasts.

Disclosure of Interests: None declared

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FRI0372 INCREASED EXPRESSION OF NOTCH RECEPTORS ON OSTEOCLAST PROGENITORS INDUCED BY RHEUMATOID ARTHRITIS

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Background: Systemic and periarticular bone loss in rheumatoid arthritis (RA) is mediated by osteoclasts, multinucleated cells originating from the myeloid lineage. Recently, Notch signaling pathway has emerged as a potential regulator of osteoclast progenitor (OCP) differentiation and activation.

Objectives: The exact role of Notch signaling in the context of arthritis is still unknown; however, its inhibition has beneficial effects in animal arthritis models. We aimed to determine the expression of Notch receptors and ligands on specific OCP subpopulations and define changes that occur in murine collagen-induced arthritis (CIA) and RA patients.

Methods: Peripheral blood, synovial tissue and subchondral bone marrow were collected from RA patients, and periarticular bone marrow (PBM) and spleen (SPL) were harvested from male C57/Bl6 mice immunized with chicken type II collagen. Notch 1 to 4 receptor expression on OCPs was analyzed by flow cytometry. Gene expression of Notch receptors/ligands was determined by qPCR from tissues and sorted OCPs. Sorted OCPs were cultured, with addition of MCSF and RANKL, in control, IgG, Jagged (Jag) 1 or Delta (DLL) 1 coated wells. Immunohistochemistry (IHC) for Notch 1 and 2 was performed on sections of murine hind paws. Research was approved by Ethics committee.

Results: We previously identified peripheral and periarticular subpopulations of murine and human OCPs, as CD45⁺CD3⁺B220⁺NK1.1⁺CD11b^{lo}/CD115⁺CCR2⁺ and CD45⁺CD3⁺CD19⁺CD56⁺CD11b⁺CD14⁺CCR2⁺ respectively, specifically associated with arthritis. Flow cytometry revealed that majority of murine splenic and periarticular OCPs express Notch 2, whereas Notch 1 and 4 were expressed on approximately 10% of cells. In CIA, this highly osteoclastogenic population is expanded as is the expression of Notch 4 in PBM and Notch 3 in SPL. Majority of human peripheral-blood OCPs express Notch 2 and 4, with a specific increase in the expression of Notch 1 and 3 in RA. In contrast, RA synovial-derived OCPs

mostly express Notch 1 to 3, whereas subchondral OCPs mostly express Notch 1 and 4. Notch ligands were analyzed at mRNA level and revealed expression of Jag1, Jag2 and DLL4 in murine sorted OCPs and Jag1 and DLL1 in human sorted OCPs. Expression of Notch 1 and 2 was confirmed by IHC on arthritic murine hind paws, with Notch 2 expressed by bone marrow, synovial tissue and chondrocytes and Notch 1 expressed by chondrocytes and synovial tissue. Increased expression of Notch 1, Notch 2 and Jag1 was also confirmed in murine arthritic periarticular tissue by qPCR. During osteoclastogenic culture, murine and human OCPs exhibit a similar gene expression pattern with higher initial expression of Notch 1 and 2, and increase in the expression of Notch 3 and 4 with differentiation. Osteoclasts were also differentiated under Notch-ligand stimulation. Coating with DLL1 results in a greater number of cells expressing osteoclast-specific TRAP, whereas Jag1 seemed to inhibit osteoclastogenesis.

Conclusion: Our results indicate that murine and human OCPs express a distinct tissue-specific pattern of Notch receptors. Notch signaling in OCPs is increased in arthritis and may contribute to the osteoclastogenic potential and increased bone resorption. Our next aim would be to determine the effect of Notch inhibition on OCP activity and arthritis severity.

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FRI0373 ASSOCIATIONS OF VASCULAR PATHOPHYSIOLOGY AND BONE METABOLISM IN ANTI-TNF- TREATED RHEUMATOID ARTHRITIS AND ANKYLOSING SPONDYLITIS PATIENTS

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Background: Cardiovascular (CV) disease and osteoporosis (OP) have become increasing challenges in the ageing population, even more in patients with inflammatory rheumatic diseases, such as rheumatoid arthritis (RA) and spondyloarthropathies. Both RA and ankylosing spondylitis (AS) have been associated with generalized and localized bone loss, accelerated atherosclerosis, increased CV morbidity and mortality.

Objectives: Bone and vascular biomarkers and parameters along with the effect of one-year anti-TNF therapy on these markers were assessed in order to determine correlations between vascular pathophysiology and bone metabolism in RA and AS.

Methods: Fifty-three patients including 36 RA patients treated with etanercept (ETN) or certolizumab pegol (CZP) and 17 AS patients treated with ETN were included in a 12-month follow-up study. Bone and vascular markers were assessed by ELISA. Bone density was assessed by DXA and quantitative CT (QCT). Flow-mediated vasodilation (FMD), common carotid intima-media thickness (ccIMT) and pulse-wave velocity (PWV) were assessed by ultrasound. The effects of vascular markers on bone and bone effects on vasculature undergone statistical analysis.

Results: Serum levels of vascular endothelial growth factor (VEGF), PDGF-BB, angiopoietin 2 (Ang2) and cathepsin K (CathK) decreased, procollagen type 1 N-propeptide (P1NP) and sclerostin (SOST) levels increased, soluble receptor activator nuclear kappa B ligand (sRANKL) and osteoprotegerin (OPG) levels showed no differences. When bone and vascular markers were correlated with each other, at baseline, OPG correlated with Ang2 and adiponectin. SOST correlated positively with ccIMT. DXA L2-4 BMD, DXA L1 BMD and DXA femoral neck (FN) BMD correlated with FMD and CRP. QCT trabecular BMD correlated with ccIMT and PON1. According to the univariate analysis, FMD correlated with OPG, ccIMT correlated with SOST and QCT trabecular BMD. Ang1, Ang2 and PDGF-BB showed correlation with Dickkopf-1 (DKK1). Ang2 also correlated with OPG. As suggested by the multivariate analysis, OPG determined FMD; DKK1 was an independent predictor of Ang1, Ang2 and PDGF-BB. OPG was a predictor of Ang2.

Conclusion: In our study of anti-TNF treated RA and AS patients, vascular and bone parameters showed numerous correlations. The therapy was clinically effective, it halted further bone loss over 1 year and reduced the production of angiogenic markers.

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FRI0374

PLASMA LEVELS OF 14-3-3 PROTEIN, S100A8/S100A9-PROTEIN, INTERLEUKIN-6, INTERLEUKIN-18, INTERLEUKIN-4, INTERLEUKIN-17, INTERLEUKIN-1B AND TUMOR NECROSIS FACTOR- α IN CHRONIC NON-BACTERIAL OSTEOMYELITIS AND NON-SYSTEMIC JUVENILE IDIOPATHIC ARTHRITIS

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Background: Chronic non-bacterial osteomyelitis (CNO) is an immune-mediated disease associated with cytokine dysbalance.

Objectives: The aim of our study was to evaluate the cytokines levels in CNO and compare to juvenile idiopathic arthritis (JIA) – disease with immune-mediated mechanism.

Methods: The diagnosis of CNO made with criteria, proposed by Jansson (2007, 2009), after the exclusion of other causes of bone disease [1]. We included 42 patients with NBO, 28 patients with non-systemic juvenile idiopathic arthritis (JIA). We evaluated plasma levels of 14-3-3 protein, S100A8/S100A9-protein, interleukine-6 (IL-6), interleukine-18 (IL-18), interleukine-4 (IL-4), interleukine-17 (IL-17), interleukine-1 β (IL-1 β) and tumor necrosis factor- α (TNF α) in 2 groups by the ELISA. Statistical analysis was carried out with Statistica 10.0 software. We utilized descriptive statistics (Me; IQR), Mann-Whitney tests.

Results: We have found differences in the proinflammatory biomarkers between CNO, JIA. Patients with NBO had lower levels of studied cytokines, exclude 14-3-3-protein, S100A8/S100A9 and interleukin-6 compare to JIA patients (table 1).

Table 1. Comparison the cytokine levels between CNO, JIA N

Parameter	NBO (n=42)	JIA (n=28)	p
Hemoglobin, g/l	112 (104; 124)	120 (114.5; 126.0)	0.02
WBC x 10 ⁹ /l	7.9 (7.0; 10.5)	8.0 (6.7; 10.0)	0.86
PLT x 10 ⁹ /l	347 (259; 408)	336.5 (274.0; 390.5)	0.98
ESR, mm/h	25.0 (9.0; 46.0)	8.5 (2.5; 13.0)	0.013
CRP, mg/l	6.1 (0.6; 2.4)	1.8 (0.4; 11.9)	0.027
14-3-3, ng/ml	21.4 (18.5; 27.1)	19.9 (18.0; 27.8)	0.77
S100A8/S100A9, ng/ml	5.9 (5.2; 6.5)	5.9 (5.0; 6.2)	0.76
IL-6, ng/ml	126.2 (112.8; 137.5)	132.4 (117.4; 142.9)	0.16
IL-18, ng/ml	270.1 (200.1; 316.1)	388.3 (373.9; 405.1)	0.0000001
IL-4, ng/ml	15.3 (11.5; 18.2)	18.7 (16.2; 20.2)	0.003
IL-17, ng/ml	83.1 (71.1; 97.3)	99.2 (87.3; 115.8)	0.003
IL-1b, ng/ml	47.4 (42.0; 51.3)	70.8 (65.3; 73.6)	0.0000001
TNF α , ng/ml	19.4 (17.8; 21.3)	23.1 (20.2; 25.9)	0.0006

Conclusion: Patients with CNO had less proinflammatory activity then JIA patients, besides IL-6 and S100A8/S100A9. Further investigations required for finding new more precise biomarkers and finding possible molecular targets for treatment.

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FRI0375

VISFATIN EFFECTS ON MSCS DURING OD VIA DIFFERENTIAL REGULATION OF LNCRNA H19 AND MICRO RNA 675-3P

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Background: Long non-coding (lnc-)RNA are regulatory molecules transcribed from DNA similar to mRNA and interact directly with DNA, RNA and proteins. Some lncRNAs have been shown to contain micro (mi-)RNAs in their sequence that can be released by splicing and lead to active miRNA molecules, e.g. lncRNA H19 includes two miRNAs 675-3p and -5p in its sequence.

Adipose tissue derived factors (adipokines) are involved in inflammation processes and osteoarthritis (OA) development. The proinflammatory adipokine visfatin has been shown to alter osteogenic differentiation (OD) of pluripotent mesenchymal stem cells (MSCs) and reduces elastic fiber expression, increases matrix mineralization and proinflammatory cytokine and chemokine production⁽¹⁾.

Objectives: We evaluated a novel effect of visfatin on lncRNA H19 in MSCs during OD. The goal was to explore the kinetics of the visfatin effect during OD with regard to H19 regulation and to investigate H19 downstream mechanisms leading to the observed altered MSC differentiation and osteoblast activity.

Methods: MSCs isolated from OA hip or knee bone (phMSC) and commercially obtained healthy human (h-)MSCs were differentiated towards osteoblasts with/without visfatin, resistin, leptin, TNF and Wnt/TGF β 1 pathway inhibitors. Supernatants were collected at days 2, 7, 9, 14 and 21 of OD, cell lysates at day 2, 7, 9, 14 and matrix mineralization assays conducted at day 21. H19 and miRNA expression was evaluated by real-time PCR after mi-RNA isolation. IL-6 was analyzed by ELISA.

Results: H19 was continuously upregulated in unstimulated controls as expected during OD but also when stimulated with other adipokines. In contrast, stimulation with visfatin significantly decreased H19 (day 2 to 14 of OD, hip-phMSCs: p = 0.0097, knee-phMSCs: p=0.0075, h-MSC: p = 0.044). Visfatin increased matrix mineralization and IL-6 production as expected (hMSC: p = 0.03, phMSC: p = 0.013)⁽¹⁾. TNF stimulation during OD did not lead to a downregulation of H19 nor increased matrix mineralization, thus showing that the effects were visfatin-dependent. H19s endogenous miRNA 675-5p was changed in parallel with H19, increased during control OD and significantly down-regulated by visfatin (e.g. day 14 p = 0.015). However, H19s endogenous miRNA 675-3p was inversely regulated, downregulated during control OD while visfatin stimulation attenuated this effect (e.g. day 14 p = 0.025). Altered Wnt-signaling and involvement of the TGF β 1 pathway could not be observed.

Conclusion: H19 is upregulated during OD and may therefore play a regulatory role in the process of osteogenesis. Visfatin stimulation of MSCs during OD showed pro-inflammatory effects, increased matrix mineralization while reducing elastic fiber production⁽¹⁾. These effects were associated with a downregulation of H19, a specific visfatin effect not triggered by other adipokines or TNF. The H19 sequence includes two endogenous micro-RNAs 675-3p and 5p. We demonstrated miRNA 675-5p to be regulated in parallel to H19, whereas miRNA 675-3p was inversely regulated and increased continuously upon visfatin stimulation. Based on these results, we hypothesize that visfatin provides a specific stimulus for the splicing of miRNA 675-3p from H19, in turn leading to H19 reduction. miRNA 675-3p thus represents an effector mechanism of visfatin that contributes to the observed functional effects in differentiating MSCs.

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FRI0376

EFFECT OF CARBAMYLATED LOW-DENSITY LIPOPROTEINS ON BONE CELLS HOMEOSTASIS

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