POS0041

THE GASOTRANSMITTER HYDROGEN SULFIDE (H₂S) IS PROTECTIVE AGAINST CALCIFIC TENDINOPATHY (CT)

I. Bernabei¹, D. Kronenberg², R. Stange², J. Bertrand³, T. Hügle¹, A. So¹, N. Busso¹, S. Nasi¹. ¹Centre Hospitalier Universitaire Vaudois and University of Lausanne, Department of Musculoskeletal Medicine, Service of Rheumatology, Lausanne, Switzerland; ²Institute of Musculoskeletal Medicine, Westfälische Wilhelms University Münster, Department of Regenerative Musculoskeletal Medicine, Münster, Germany; ³Otto-von-Guericke University, Department of Orthopaedic Surgery, Magdeburg, Germany

Background: Pathological (or heterotopic) calcification is the deposition of calcium-containing crystals in soft tissues that normally do not calcify. The deposition of these crystals in tendons such as the rotator cuff and the Achilles tendon is known as calcific tendinopathy (CT). CT is a painful condition, which increases tendon rupture rate and leads to disability.

Objectives: To understand what inhibits calcification, in order to provide new strategies to treat a condition for which existing therapies are ineffective.

Methods: We investigated the role of the gasotransmitter hydrogen sulfide (H₂S), and in particular of the H₂S-producing enzyme cystathionine γ -lyase (CSE) in CT. *In vitro*, we induced calcification in tenocytes from WT and CSE KO mice or we treated WT tenocytes with different H₂S donors. *In vivo*, calcification was assessed in a surgery-induced murine model of CT (tenotomy of the Achilles tendon) and in a spontaneous model of CT (aging). Samples obtained from patients with rotator cuff or Achilles tendon CT were also analyzed. To investigate the underlying mechanisms of the CSE-H₂S effect, we focused on the bone morphogenic proteins (BMPs) pathway. We additionally explored if altered extracellular matrix (ECM) organization, due to lysyl oxidase (LOX) activity and aberrant collagen-crosslinks, could also be involved in CT. In this context, we studied if H₂S could affect LOX expression and activity.

Results: *In vitro*, tenocyte calcification was inhibited by exogenous H₂S-donors, while it was exacerbated in CSE KO tenocytes. The protective role of CSE-H₂S was confirmed *in vivo*. In aged mice, microtomography analysis revealed exacerbated Achilles tendon calcification in CSE KO mice compared to WT. In the surgery-induced model of CT, an inverse correlation between calcification and CSE expression in operated Achilles tendon was seen over time. Similarly, inverted correlation between calcification and CSE expression was found in human CT samples.

Reduced calcification in tenocytes exposed to H₂S was accompanied by decreased expression of genes coding for BMP2, BMP4 and decreased activation of the BMP signaling pathway (pSMAD1/5/8). On the contrary, BMPs expression and BMPs-pathway activation were exacerbated in CSE KO tenocytes compared to WT tenocytes.

We next investigated whether ECM disorganization could play a role in CT. Tenocytes cultured in calcification media and treated with the pan-inhibitor of lysyl oxidases (LOX, LOXL1-4) β -aminopropionitrile (BAPN) showed decreased calcification. This pointed to a potential beneficial role of LOX inhibition, therefore decreased collagen-crosslinks, in CT. By analysis of LOXs gene expression in WT and CSE KO tenocytes cultured in calcifying condition, we found much higher expression (4-fold) of LOX, LOXL2 and LOXL4 in CSE KO tenocytes. Moreover, H₂S-donors inhibited LOX activity. Altogether, these results suggest that decreased H₂S could lead to aberrant LOX expression and activity, excessive collagen cross-links in the ECM, and ultimately calcification. Further experiments are ongoing to prove these hypotheses.

Conclusion: We suggest targeting H₂S production by CSE, or supplying an H₂S-donor, is of therapeutic relevance to pathological calcification in the context of CT and can modify its disease course.

The anti-mineralizing effect of H₂S in tendons could be due to both inhibition of the BMPs pathway and suppression of abnormal LOXs activity.

Disclosure of Interests: None declared.

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POS0042

NOTCH 1 INHIBITION INCREASES OSTEOCLAST PROGENITOR ACTIVITY IN THE MOUSE MODEL OF RHEUMATOID ARTHRITIS

M. Filipović¹, A. Šućur¹, D. Flegar¹, Z. Jajić², M. Ikić Matijašević³, N. Lukač¹, N. Kovačić¹, T. Kelava¹, D. Šiš¹, K. Zrinski Petrović¹, V. Katavić¹, D. Grčević¹.

¹University of Zagreb School of Medicine, Croatian Institute for Brain Research, Laboratory for Molecular Immunology, Zagreb, Croatia; ²University of Zagreb School of Medicine, Clinical Hospital Center "Sestre Milosrdnice", Department of Rheumatology, Physical Medicine and Rehabilitation, Zagreb, Croatia; ³University of Zagreb School of Medicine, Clinical Hospital "Sveti Duh", Department of Clinical Immunology and Allergology, Zagreb, Croatia

Background: Osteoclasts mediate periarticular and systemic bone loss in rheumatoid arthritis (RA). Osteoclast progenitor cells (OCPs) derived from the

myeloid lineage are susceptible to regulation through Notch signaling. Murine bone marrow and splenic OCPs, identified as CD45⁺Ly6G⁺CD3⁺B220⁺NK1.1⁺CD11b^{lo}CD115⁺CCR2⁺ cells, are specifically increased in arthritis. We previously identified an increased frequency of OCPs expressing Notch receptors in arthritic mice.

Objectives: Several studies suggested that Notch signaling modulation affects the course of experimental arthritis. We aimed to determine the effects of Notch receptor signaling inhibition on OCP activity and arthritis severity in murine collagen-induced arthritis (CIA).

Methods: Male C57/BL6 and DBA mice were immunized with chicken type II collagen and treated with i.p. injections of anti-Notch 1 neutralizing antibodies (1mg/kg). Notch receptor 1 through 4 expression on OCPs was analyzed by flow cytometry in periarticular bone marrow (PBM) and spleen (SPL). Gene expression of Notch receptors, ligands and transcription targets as well as osteoclast differentiation genes RANK, cFos and cFms was determined by qPCR from tissues and sorted OCPs. FACS sorted OCPs were stimulated by osteoclastogenic factors (M-CSF and RANKL), in control, IgG, Jagged (Jag)1 or Delta-like (DLL)1 coated wells, with or without anti-Notch 1 antibodies. Research was approved by the Ethics Committee.

Results: We confirmed the expression of Notch receptors on OCPs by flow cytometry with Notch 1 and 2 being most abundantly expressed (around 25% and 40% positive OCPs in PBM and 35% and 20% in SPL respectively), with a significant increase of Notch 2 expression in arthritis. Seeding OCPs on DLL1 coated wells significantly increased while seeding on Jag1 coated wells significantly decreased osteoclastogenesis as reflected on the number of TRAP⁺ osteoclasts and expression of osteoclast differentiation genes. The addition of anti-Notch 1 antibodies to ligand-stimulated OCPs resulted in an increased number of TRAP⁺ osteoclasts, partially reversing Jag1 inhibition. *In vivo* treatment with anti-Notch 1 antibodies did not affect total OCP frequency, but increased expression of Notch 4 both in PBM and SPL as seen by flow cytometry and qPCR. Additionally, anti-Notch 1 treatment stimulated Notch transcription factors HES and HEY. Both PBM and SPL cultured OCPs from anti-Notch 1 treated mice produced a higher number of large TRAP⁺ osteoclasts, doubling the area covered with osteoclasts in the latter compared to untreated mice. Increased osteoclastogenesis *in vitro* was further confirmed by an increased expression of osteoclast differentiation genes in the treated group.

Conclusion: Our results confirm that Notch signaling may represent an important therapeutic target for the regulation of osteoclast activity in arthritis. Both *in vitro* and *in vivo* anti-Notch 1 neutralizing antibodies enhanced osteoclastogenesis in CIA model, implying an inhibitory role of Notch 1 signaling in osteoclast differentiation. As Notch 2 expression is increased on OCPs of arthritic mice, we next plan to determine the effects of Notch 2 neutralization on osteoclast activity and arthritis severity.

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POS0043

PERIPHERAL QUANTITATIVE COMPUTED TOMOGRAPHY IN THE ASSESSMENT OF BONE MINERAL DENSITY IN ANTI-TNF-TREATED RHEUMATOID ARTHRITIS AND ANKYLOSING SPONDYLITIS PATIENTS

B. Juhász¹, K. Gulyás², Á. Horváth², E. Végh², A. Pusztai², A. Szentpetery³, Z. Pethő², N. Bodnár², A. Hamar², L. Bodoki², H. P. Bhattoa⁴, É. Szekanecz¹, K. Hodosi², A. Domjan², S. Szamosi², C. Horváth⁵, S. Szántó², G. Szűcs², H. Raterman⁶, W. Lems⁷, O. Fitzgerald⁸, Z. Szekanecz². ¹University of Debrecen, Oncology, Debrecen, Hungary; ²University of Debrecen, Rheumatology, Debrecen, Hungary; ³Uppsala University Hospital, Rheumatology, Uppsala, Sweden; ⁴University of Debrecen, Laboratory Medicine, Debrecen, Hungary; ⁵Semmelweis University, Medicine, Budapest, Hungary; ⁶Northwest Clinics, Rheumatology, Alkmaar, Netherlands; ⁷University of Amsterdam, Rheumatology, Amsterdam, Netherlands; ⁸University College Dublin, Conway Institute for Biomolecular Research, Dublin, Ireland

Background: Rheumatoid arthritis (RA) and ankylosing spondylitis (AS) have been associated with osteoporosis. There have been very few data on the use of peripheral quantitative computed tomography (QCT) in anti-TNF-treated patients.