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HOT TOPICS

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HT1

High Serum Levels and Liver Expression of Sclerostin in Patients with Primary Biliary Cirrhosis. Association with Markers of Bone Remodelling and Severity of Cholangitis

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Sclerostin is involved in the regulation of osteoblastogenesis and little is known about its role in the development of bone disease in primary biliary cirrhosis (PBC), characterised by low bone formation. Therefore, we have assessed the circulating levels and the liver expression of sclerostin in this cholestatic disease. Serum sclerostin levels were measured in 83 women with PBC (mean age: 60±12 years) and 101 control women. Lumbar and femoral BMD as well as parameters of mineral metabolism and bone remodeling were measured. Moreover, sclerostin gene expression in the liver was assessed in samples of liver tissue taken by biopsy in 11 PBC patients and 5 healthy controls by real time PCR, and presence and distribution of sclerostin was evaluated in liver slices from 11 patients by immunohistochemistry. The presence and severity of histologic lesions were assessed semiquantitatively in the same liver samples. 77% of patients had low BMD (22% osteoporosis and 55% osteopenia). PBC patients had higher sclerostin levels than controls (76.7±38.6 vs. 32.5±14.7 pmol/L, $p<0.001$). Serum sclerostin correlated inversely with markers of bone formation and resorption. Sclerostin mRNA in the liver was overexpressed as compared with control samples (2.7±0.3 fold vs healthy liver). Sclerostin was detected by immunohistochemistry in 7 of the 11 liver samples and mainly located in the bile ducts. Sclerostin was associated with the severity of cholangitis ($p=0.02$) and indirectly with the degree of lobular inflammation ($p=0.03$). Sclerostin mRNA expression was higher in samples positive by immunohistochemistry (2.9±0.4 vs 2.5±0.3, p:n.s.), and particularly in those with lobular granuloma (3.6±0.6 vs 2.4±0.2, $p=0.02$). The increased expression of sclerostin in the liver and the association with histologic cholangitis may explain the high serum levels of this protein in patients with PBC, thus suggesting that sclerostin influences the decreased bone formation in this cholestatic disease.
Disclosure: The authors declared no competing interests.

HT2

Osteocyte-Specific Ablation of Pparγ Results in Sost Down-Regulation and Increased Periosteal Bone Formation but Decreased Bone Turnover in Mice

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While heterozygous Pparγ-deficient mice exhibited high bone mass with increased osteoblastogenesis from bone marrow progenitors, the role of Pparγ specifically in late osteoblast/osteocyte (ocy) is not yet properly understood. We crossed Pparγ-loxP with Dmp1-cre mice to generate Ocy-Pparγ$^{-/-}$ and -Pparγ$^{+/+}$ male mice, which developed normally until 3 months of age, at which time they were analyzed in details. Tissue and cell specificity of Pparγ deletion were confirmed respectively by western blot and immunostaining. Total body lean & fat mass as well as handgrip strength were comparable in Ocy-Pparγ$^{-/-}$ and -Pparγ$^{+/+}$ mice. Femoral BMD was significantly higher in Ocy-Pparγ$^{-/-}$ (78.6±1.3 vs 73.4±2.1 mg/cm^2 in Pparγ$^{+/+}$, $p<0.001$). Trabecular and cortical microarchitecture respectively evaluated at the distal and midshaft femur was improved in Ocy-Pparγ$^{-/-}$: BV/TV +28.6%, TbTh +12.5%, CtTV +9.8%, CtBV +12.0% and CtTh +4.5% compared to Pparγ$^{+/+}$ (all $p<0.05$). Periosteal bone forming rate was higher in Ocy-Pparγ$^{-/-}$ (+66.5% vs Pparγ$^{+/+}$, $p<0.05$), whereas no significant differences were observed at endocortical surfaces. In contrast CTx was decreased in Ocy-Pparγ$^{-/-}$ (8.6±0.6 vs 12.9±0.5 ng/ml in Pparγ$^{+/+}$, $p<0.001$). Gene expression analyses in the osteocytic fraction of cells extracted from the femur showed lower *Sost* mRNA levels, 50% lower in Ocy-Pparγ$^{-/-}$ compared with Pparγ$^{+/+}$, whereas *Opg* or *RankL* mRNA levels were comparable. However, in the osteoblastic fraction, both *Runx2* and *Opg* levels were significantly higher in Ocy-Pparγ$^{-/-}$, respectively +19.2% and +197.6% vs Pparγ$^{+/+}$, both $p<0.01$. In conclusions, Ocy-specific ablation of Pparγ down-regulates *Sost* and upregulates *Opg* expression, resulting in increased periosteal bone formation but lower bone turnover and high bone mass. These observations suggest a role of Pparγ in osteocytes on the control of bone modelling and remodelling by these cells *in vivo*.
Disclosure: The authors declared no competing interests.

HT3

Intensive Bisphosphonate Therapy Increases the Risk of Fracture and Requirement for Orthopaedic Surgery in Paget's Disease: the PRISM-EZ Study

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The optimal management strategy for Paget's disease of bone (PDB) remains to be established although recent clinical guidelines have suggested that bisphosphonates should be given to maintain alkaline phosphatase (ALP) concentrations within the mid reference range. We report upon the long term outcome of treatment in PDB within the PRISM-extension with Zoledronic acid study (PRISM-EZ) in which 502 PDB patients, who all participated in the PRISM study, were followed for an additional 3 years. There were two treatment groups; symptomatic therapy (n=232) where bisphosphonates were only given if symptoms were present and intensive therapy (n=270) where bisphosphonates were given with the aim of maintaining ALP within the reference range. Zoledronic acid was the bisphosphonate of choice in the intensive group. Mean concentrations of ALP were in the mid-reference range in the intensive group throughout the study (mean ± SD normalised ALP = 0.71±0.30) but at the upper end of the reference range in the symptomatic group (1.01±0.81; p<0.001 between groups). There was no significant difference between treatment groups in bone pain and quality of life scores assessed by the SF36 questionnaire. Thirty-eight patients suffered a fracture (23 in the intensive and 15 in the symptomatic treatment groups, respectively). Six out of the above 38 patients had fracture of bone affected by PDB. Sixteen patients required orthopaedic surgery. Patients randomised to receive intensive bisphosphonate therapy were more likely to experience a fracture or undergo orthopaedic surgery than those in the symptomatic arm (Hazard ratio 1.94, 95% CI 1.07-3.51, p=0.029) and this remained significant after correction for baseline characteristics (HR 1.85, 95% CI 1.02-3.38, p=0.044]. We conclude that intensive bisphosphonate therapy confers no benefit in patients with PDB and on the contrary may be harmful. Treatment should be directed at patient symptoms rather than keeping ALP concentrations within the reference range.

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HT4

Inhibition of Vascular Calcification by Extracellular Nucleotides, P2 Receptors and NPP1

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Vascular calcification (VC) shares some similarities to skeletal mineralisation, and involves hydroxyapatite deposition in arteries and cardiac muscle. Whilst VC has severe clinical consequences, the cellular mechanisms responsible are not fully elucidated. ATP and UTP (≥1μM) inhibit bone mineralisation via P2 receptor-dependent (P2R) and independent mechanisms. The latter involves the hydrolysis of extracellular nucleotides by NPP1 to produce pyrophosphate (PP_i), a key mineralisation inhibitor. This study investigated whether extracellular nucleotides also regulate VC. Vascular smooth muscle cells (VSMCs) were cultured in calcifying medium containing 2.5 mM phosphate for 14 days. We found that VSMCs express multiple P2Rs and expression was up-regulated in calcifying conditions. The key source of extracellular ATP is controlled release from cells: removal of endogenous ATP by apyrase (an ecto-nucleotidase which hydrolyses ATP) resulted in a 45% increase in VSMC calcification. Culture with exogenous ATP and UTP (≥1 μM) decreased VSMC calcification by ≤80% and 90%, respectively (p<0.001). The selective agonists, 2-thioUTP and MRS2768, also inhibited VSMC calcification by <70% (p<0.001) suggesting a role for the $P2Y_2R$ in mediating these effects, Furthermore, the level of calcification is increased 2-fold in VSMCs from $P2Y_2R$ knockout mice. VC is associated with increased VSMC apoptosis and a transdifferentiation of VSMCs towards the osteogenic lineage. We observed that ATP/UTP increased VSMC number and decreased the expression of genes associated with osteoblast differentiation (e.g. Runx2, osterix, Ocn). CTP and GTP (≥10μM), which are not P2R agonists but are hydrolysed by NPP1 to produce PP_i, blocked VSMC calcification by ≤70%. Furthermore, in NPP1 knockout VSMCs, the inhibitory actions of ATP and UTP were 10-fold less potent. These results indicate the P2R-independent mechanisms (involving PP_i) contribute significantly to the inhibitory actions of extracellular nucleotides on VC. Taken together, our data suggest an important role for extracellular nucleotides, the $P2Y_2R$ and NPP1 in the regulation of VC.

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HT5

Abstract not available

HT6

High Periostin Levels in Cortical Bone of Cathepsin K Knock-Out Mice are Responsible for Increased Periosteal Bone Formation and Bone Mass

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Cathepsin K (CatK) inhibition in preclinical models results not only in lower bone resorption but also in higher bone formation (BF) on both remodeling and modeling surfaces. The mechanisms for greater BF at modeling surfaces remain unexplained. *In vitro* data suggest that periostin is proteolytically degraded by CatK. Periostin (Postn) is a matricellular protein expressed in the periosteum and osteocytes that mediates β-catenin signalling and skeletal response to loading. We hypothesised that higher BF in *Ctsk*$^{-/-}$ mice might be related to increased periostin expression. For this purpose, BMD, microstructure and histomorphometry were evaluated in the progeny of a *Postn*$^{+/-}$ X *Ctsk*$^{+/-}$ mouse cross. Postn immunostaining was more intense in *Ctsk*$^{-/-}$ osteocytes and periosteum surfaces vs WT. *Postn*$^{+/+}$;*Ctsk*$^{-/-}$ have higher BMD, BV/TV, CtBV, CtTh, whereas *Postn*$^{-/-}$;*Ctsk*$^{+/+}$ have lower femoral BMD, CtBV, and CtTh compared to WT. *Postn*$^{+/+}$;*Ctsk*$^{-/-}$ have higher Ps-MAR and –BFR (+58% and + 137% vs WT, $p<0.05$), as well as Ec-MAR (+74% vs WT, $p<0.05$) but lower Ec-MPm/BPm (-40% vs WT, $p<0.05$), indicating that BF is increased on both cortical envelopes and independently of remodeling surfaces. Removing both Postn alleles in *Ctsk*$^{-/-}$ mice, i.e. *Postn*$^{-/-}$;*Ctsk*$^{-/-}$, prevented the increase in CtBV and Ps-BFR, but had no effect on Ec-MPm/BPm which remained low (-51% vs WT, $p<0.05$), indicating that Postn mediates cortical bone formation but has no influence on CatK dependent bone remodeling. In contrast, trabecular microarchitecture at the distal femur and vertebrae was not affected by Postn deletion in *Ctsk*$^{-/-}$ mice, indicating that Postn-dependent bone formation in these mice occurs specifically at cortical surfaces. In conclusions, periostin expression is increased in *Cstk*$^{-/-}$ mice and is responsible for the increased modelling-based bone formation observed on cortical bone in the absence of cathepsin K.

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