

Bone Research Society Meeting

3–5 July 2007, Aberdeen, UK

Claire E Clarkin[†] & Nicholas C Harvey

[†]Author for correspondence

Department of Veterinary Basic Sciences, The Royal Veterinary College, Royal College Street, London, NW1 0TU, UK

Tel.: +44 207 468 5036; Fax: +44 207 468 5204; cclarkin@rvc.ac.uk

The Bone Research Society (BRS) is one of the premier research societies in the bone field, and brings together top scientists and clinicians from the UK and around the world. Indeed the 2007 annual meeting in Aberdeen was an excellent example of the opportunities afforded by the society for interaction between basic and clinical scientists. The meeting covered many exciting areas, and significant topics included bone pain, vesicular trafficking and bone signaling, bone changes in inflammatory arthritis and new therapeutic advances in osteoporosis. These areas will be expanded upon below, in reports written by young investigators in the Oliver Bird PhD Studentship Scheme.

Bone pain

Whyte L, Taylor A, Sham C, Lowes C, Huesa C, Halliday G (University of Aberdeen, UK)

This first session of the meeting examined the role of cannabinoids and acid in the pathogenesis of bone pain. The importance of local pH on bone turnover was elucidated by Tim Arnett (University College London, UK). Increasing the acidity of the extracellular environment was previously thought to influence physico-chemical dissolution of the bone mineral. Arnett explained, however, that osteoclast resorption *in vitro* at low pH is calcitonin sensitive and therefore osteoclast mediated. TRPV1 is an acid-sensing receptor, and was shown to be present in osteoclasts and upregulated by low pH [1]. As a result, TRPV1 could act as a modulator of osteoclast function

and hence provide a possible target for the development of novel therapeutic agents that can block TRPV1 action to decrease bone resorption.

Ruth Ross (University of Aberdeen, UK) presented a detailed overview of the endocannabinoid system. This system is regulated by the ligands anandamide and 2-arachidonyl-glycerol (2-AG). These compounds, along with plant derived cannabinoids (phytocannabinoids) and synthetic analogues elicit their effects through differentially expressed G-protein coupled receptors, designated CB₁ and CB₂. CB₁ is highly expressed throughout the CNS and some peripheral tissues, while CB₂ is primarily located in immune cells [2]. Rob van't Hof (University of Edinburgh, UK) described the expression of both CB₁ and CB₂ in bone marrow cells, osteoclasts and osteoblasts, with CB₂ being the most highly expressed. The enzymes responsible for endocannabinoid synthesis and breakdown have now also been detected in bone cells by the Edinburgh group. Such findings, together with data presented by Susan Ridge (University of Aberdeen, UK) that indicated the presence of anandamide and 2-AG in human osteoblasts and osteoclasts, highlight a potential direct role of endocannabinoids in regulating bone cell function. van't Hof additionally summarized previously published data on CB₁ and CB₂ knockout mice, which have a high bone mass and low bone mass phenotype, respectively [3,4]. These studies suggest that CB₂ receptors are expressed on cells within the bone microenvironment, and thus a

direct influence of cannabinoid signaling in bone. However, the mechanism underlying bone turnover regulation by CB₁ via the CNS (through neurogenic relay) remains largely unexplored and may benefit from the use of conditional knockout animal studies in the future.

Vesicular Trafficking & Bone Disease

Baldwin H, Falconer J, Jefferson M, Kirmizidis G, Lindop J, Wilson A (University of Newcastle upon Tyne, UK)

Osteoclast vesicular trafficking defects have been implicated in diseases including osteopetrosis, some skeletal dysplasias and Huntington's Chorea [5–7]. The ruffled border and functional secretory domains of the osteoclast are uniquely dependent on vesicular trafficking of acidic vesicles. Vesicles maintain low pH at the resorbing site while releasing a plethora of proteases, which are able to degrade the bone matrix. Breakdown products of bone resorption are then endocytosed and secreted through the functional secretory domain.

Fraser Coxon (University of Aberdeen, UK) discussed the Rab family of proteins, which, through their GTPase activity, are required for the coordination of vesicular trafficking [8]. Two members of this family in particular are thought to play a vital role in the osteoclast behavior; Rab3D is essential for post-TGN-vesicle trafficking, while Rab7 regulates transport of late endosomes to the ruffled border where it colocalises with the PLEKHM1 protein. Loss of function mutations in PLEKHM1 result in a lack of ruffled border formation, which consequently reduces bone resorption, causing an osteopetrotic phenotype. This further demonstrates the importance of the Rab family in vesicular regulation and their potential importance as drug targets for osteopetrosis [5].

Uwe Kornak (Institute for Medical Genetics, Berlin, Germany) showed that the loss of the endosome chloride channel, CIC-7, impairs endocytosis in the osteoclast and also causes osteopetrosis. CIC-7 acts in conjunction with the H⁺-V-ATPase, mediating acidification of lysosomes and the ruffled border [9]. These findings show the potential of functional genetics to give new insights into disease. Morten Karsdal (Nordic Bioscience, Herlev, Denmark) also talked on the importance of CIC-7 chloride channels and the possibility of targeting them as a therapy for osteoporosis and other diseases of abnormal bone loss. This is an exciting prospect as it aims to uncouple bone resorption from formation and provide a mechanism by which to restore the balance between the two [10]. Debbie Scott (University of Aberdeen, UK) received a New Investigator Award from the BRS and took the opportunity to present recent data indicating that patients with osteoclast-poor osteopetrosis had mutations in RANKL, a potential cause of this disease. Fiona Brunton (Queen Mary's University London, UK) discussed the possibility of targeting osteoclasts via the $\alpha_v\beta_3$ integrin, which mediates cell adhesion to extracellular matrix, as a potential treatment for bone loss in rheumatoid arthritis (RA).

Signalling pathways & bone formation

Roberts A, Pringle S, Shields A, Brunton F, Kamperidis P (Kings College London and Queen Mary's University London, UK)

The control of bone remodelling homeostasis via the autonomic nervous system was presented by Florent Elefteriou (Vanderbilt University Medical Centre, TN, USA). It is known that obese individuals are less likely to develop osteoporosis, while postmenopausal women frequently display decreased bone mass. This suggests that body weight, bone mass and the reproductive system may be regulated, at least in part, by similar signaling pathways [11].

Leptin is a protein hormone with important effects in regulating body weight, metabolism and reproductive

function. Data from leptin knockout mice, which exhibit increased bone mass, demonstrated that central injection of leptin was unable to restore the phenotype of a circulatory conjoined mouse. This suggests that hypothalamic neurons, and not soluble factors, are capable of modulating bone homeostasis. These data are supported by the finding that adrenergic receptors are required for the anti-osteogenic effects of leptin. Adrenergic receptors bind adrenergic agonists such as the sympathetic neurotransmitter norepinephrine and the circulating hormone epinephrine [12]. β_2 -adrenoceptors are expressed by osteoblasts, suggesting that β -blockers may provide a novel method for treatment for osteoporosis. However, clinical data remain inconsistent and thus the evidence does not currently support the use of β -blockers to reduce fracture risk.

Matthew Brown (University of Queensland, Australia) described the discovery and characterisation of the gene responsible for fibrodysplasia ossificans progressiva (FOP), namely activin receptor type I (ACVR1) [13]. FOP is a hereditary disease characterized by post-traumatic ossification of soft tissues. Recent advances in genomic analysis have enabled studies of afflicted families, which have identified regions of chromosomes 2, 4 and 6 as linked to disease phenotype. Further analysis highlighted a non-synonymous point mutation in the glycine-serine (GS) domain of ACVR1, which is required for the regulation of receptor activation and signaling. The R206H mutation is present in all classical FOP cases and it is this destabilization of the GS domain that is believed to be the underlying cause of the ectopic osteogenesis observed in FOP. Further exploration of the downstream signalling from ACVR1, and the development of a mouse model for FOP, may enable novel therapeutics to be developed for this condition.

Wendy Balemans (University of Antwerp, Belgium) concluded the session and reported her work investigating the interaction of sclerostin (produced by the *SOST* gene) with the Wnt and bone

morphogenetic protein (BMP) signaling pathways. *SOST* was identified through genetic linkage studies on patients with Van Buchem disease and sclerosteosis, in which osteocytes are deficient in sclerostin. Sclerostin blocks bone formation, and shows homology to canonical BMP antagonists, such as the DAN family and Wise, and is capable of inhibiting late BMP responses. However, it does not block early BMP responses, or demonstrate inhibition of direct BMP target genes, suggesting an indirect mechanism. It was subsequently shown that sclerostin binds directly to LRP5 and LRP6, co-receptors for the Wnt receptor Frizzled, which act downstream of BMP signaling in bone. Therefore, sclerostin is thought to inhibit bone formation by directly targeting the crosstalk between BMP and Wnt signaling in osteoblast maturation [14].

Bone loss & inflammatory arthritis **Blair P, Clarke L, Pericleous C, Sala E, Stock C (University College London, London, UK)**

The bone loss and inflammatory arthritis session focused on the importance of cytokines and growth factors within the microenvironment of the inflamed synovium. The mixture of factors within the joint predisposes to local bone resorption, and thus erosion formation and destruction of the joint. The clinically important message from these presentations was that bone erosion and synovitis may be independently regulated, which has implications for new therapeutic interventions. Secondly, synovitis may not be detectable in the clinic; this is born out by radiological studies, presented by Paul Emery (University of Leeds, UK), showing that patients with RA who are in clinical remission may still develop erosive change because of occult synovitis only detectable on magnetic resonance imaging [15].

Steven Goldring (Cornell University, NY, USA) stressed the importance of nuclear factor (NF)- κ B ligand (RANKL), produced by activated T cells in the inflamed synovium, in regulating

the formation and activation of osteoclasts: the BxN/RANKL knockout mouse does not form osteoclasts, but does develop inflammatory arthritis with no erosive change. Expression of RANKL is high at sites of bone erosion, with low levels of osteoprotegerin (OPG – a RANKL antagonist) [16]. Goldring also discussed the importance of the Wnt signaling pathway: in inflamed joints the up-regulation of TNF- α increases dickkopf-1 (dkk-1) expression, which, by preventing osteoblast differentiation via Wnt, increases osteoclast differentiation leading to bone erosion [17].

Matthew Gillespie (St Vincent's Institute, Australia) introduced the emerging field of osteoimmunology, investigating the interaction between the immune system and bone cells. He highlighted a number of key regulatory factors shared by both groups, including T-cell derived RANKL. T cells also secrete proresorptive cytokines including IL-1, -6 and -17, which further stimulate RANKL expression. Conversely T cells also express osteoclast inhibitors, such as IFN- γ , OPG and sFRP-1, a Wnt decoy receptor. IL-12, -18 and -23 act in a positive feedback loop mechanism to promote T-cell dependent osteoclast inhibition [18]. T-cell and bone cell interactions are further highlighted by the involvement of C-type lectins, CD69, OCIL and Nkrps, all of which are markers of activated T cells and are known to inhibit osteoclastogenesis. However, their mode of action is still poorly understood [19].

Alongside targeting the mediators of bone degradation, dysregulation of the cells that produce these cytokines may be important in disease. Berent Prakken (Utrecht University, The Netherlands) highlighted the role of T cells, in particular regulatory T cells (Tregs) in RA and juvenile idiopathic arthritis (JIA). In experimental animal models of autoimmunity Tregs are critical for the prevention and treatment of disease. Patient data indicate that there may be insufficient numbers of functional Tregs in autoimmune disease; although there are a large number of Tregs at the site of inflammation in RA, they are not protective. However, autoreactive T cells

against heat shock protein (hsp)60, a highly conserved, immune dominant stress protein released upon tissue injury and inflammation, are associated with good prognosis in JIA. A study of immunotherapy using hsp60 showed a good response in RA patients [20].

New therapeutic issues

King V, Anderson E, Ballantine L, McGrath M, Patakas A, Thalhamer T (Department of Immunology, Infection & Inflammation, University of Glasgow, UK)

Bisphosphonates are a class of drugs that inhibit resorption of bone by modifying normal osteoclast function. This session detailed their use in cancer-related bone disease, as well as new evidence regarding treatment regimens in Paget's disease of bone. The final talk elucidated exciting new developments in molecular based therapies for osteoporosis.

Robert Coleman (Weston Park Hospital, Sheffield, UK) discussed strategies for improving bone health in cancer patients using bisphosphonates [21]. Cancer may have a direct influence on bone locally as a result of metastases, but newer treatments, such as aromatase inhibitors for breast cancer, have been shown to reduce bone density and predispose to fracture. Bisphosphonates, mainly pamidronate and zoledronate, have been found effective in several clinical trials at reducing the burden associated with metastatic disease, with zoledronate being more potent. Recent studies have suggested a synergistic effect of bisphosphonates with chemotherapeutic agents in preventing metastatic change. The bone adverse nature of treatments such as aromatase inhibitors means that fracture risk and bone mineral density assessment should be considered early on in treatment.

Bisphosphonates can also be used in the treatment of Paget's disease of bone. This condition is characterized by abnormal bone turnover leading to bone pain, deformities and increased fracture risk [22]. Stuart Ralston (University of Edinburgh, UK) presented the results from the PRISM study, a large

randomized controlled trial that compared symptomatic (bisphosphonates only given when bone pain is present) with intensive (bisphosphonates given to normalize alkaline phosphatase) treatments [23]. The study revealed that while intensive treatment normalized alkaline phosphatase levels in 80% of cases, this was not reflected in any improvement in quality of life, risk of fracture or hearing loss. Thus, at the current time, symptomatic treatment of patients with Paget's disease appears to be the most appropriate solution.

Alternative therapeutic approaches in the management of osteoporosis were discussed by Graham Russell (Oxford University Institute of Musculoskeletal Sciences, UK). At present bisphosphonates, selective estrogen receptor modulators and strontium ranelate are the most commonly used treatments. Novel alternatives include calcylitics, which stimulate endogenous parathyroid hormone production, cathepsin K inhibitors, which act by selectively suppressing bone resorption, and RANKL antibodies, which inhibit osteoclast formation. Studies have also demonstrated the efficacy of antibody blockade of SOST, which leads to an increase in bone mass. Furthermore molecular signalling pathways such as the BMP and Wnt/LRP5/6 cascade [24] may be potential future therapeutic targets.

Conclusion

The 2007 BRS meeting was fully consistent with the society's aim to be at the forefront of basic and clinical bone science, and was extremely successful in combining these two branches together in a cohesive and high quality meeting. It highlighted many significant advances in bone research that have occurred over the last year involving many exciting research outcomes and the subsequent progression of therapeutic options. In addition, there has been identification of new genes that play critical roles in regulating and maintaining bone homeostasis and these will undoubtedly prove highly valuable when investigating the mechanisms involved in the initiation of bone dysfunction in the future.

Financial & competing interests disclosure

The author has no relevant affiliations or financial involvement with any organization or entity with a financial interest in or financial conflict with the subject matter or materials discussed in the manuscript. This includes

employment, consultancies, honoraria, stock ownership or options, expert testimony, grants or patents received or pending, or royalties.

No writing assistance was utilized in the production of this manuscript.

Bibliography

1. Arnett T: Regulation of bone cell function by acid–base balance. *Proc. Nutr. Soc.* 62, 511–520 (2003).
2. Ross RA: Anandamide and vanilloid TRPV1 receptors. *Br. J. Pharmacol.* 140, 790–801 (2003).
3. Idris AI, van't Hof RJ, Greig IR *et al.*: Regulation of bone mass, bone loss and osteoclast activity by cannabinoid receptors. *Nat. Med.* 11(7), 774–779 (2005).
4. Ofek O, Karsak M, LeClerc N *et al.*: Peripheral cannabinoid receptor, CB2, regulates bone mass. *Proc. Natl Acad. Sci. USA* 103(3), 696–701 (2005).
5. Van Wesenbeeck L, Odgren PR, Coxon FP, *et al.*: Involvement of PLEKHM1 in osteoclastic vesicular transport and osteopetrosis in incisors absent rats and humans. *J. Clin. Invest.* 117, 919–930 (2007).
6. Truant R, Atwal R, Burtnik A: Hypothesis: huntingtin may function in membrane association and vesicular trafficking. *Biochem. Cell Biol.* 84, 912–917 (2006).
7. Boyadjiev SA, Fromme JC, Ben J *et al.*: Cranio-lenticulo-sutural dysplasia is caused by a SEC23A mutation leading to abnormal endoplasmic-reticulum-to-Golgi trafficking. *Nat. Genet.* 38, 1192–1197 (2006).
8. Cai H, Reinisch K, Ferro-Novick S: Coats, tethers, Rabs and SNAREs work together to mediate the intracellular destination of a transport vesicle. *Dev. Cell* 12, 671–682 (2007).
9. Kornak U, Kasper D, Bösl MR *et al.*: Loss of the CIC-7 chloride channel leads to osteopetrosis in mice and man. *Cell* 104, 205–215 (2001).
10. Henriksen K, Gram J, Schaller S *et al.*: Characterization of osteoclasts from patients harboring a G215R mutation in CIC-7 causing autosomal dominant osteopetrosis type II. *Am. J. Path.* 164, 1537–1545 (2004).
11. Takeda S, Eleftheriou F, Karsenty G: Common endocrine control of body weight, reproduction, and bone mass. *Annu. Rev. Nutr.* 23, 403–411 (2003).
12. Eleftheriou F, Ahn JD, Takeda S *et al.*: Leptin regulation of bone resorption by the sympathetic nervous system and CART. *Nature* 434(7032), 514–520 (2005).
13. Shore EM, Xu M, Feldman GJ *et al.*: A recurrent mutation in the BMP type I receptor ACVR1 causes inherited and sporadic fibrodysplasia ossificans progressiva. *Nat. Genet.* 38(5) 525–527 (2006).
14. Balemans W, Devogelaer JP, Cleiren E, Piters E, Caussin E, Van Hul W: Novel LRP5 missense mutation in a patient with a high bone mass phenotype results in decreased DKK1-mediated inhibition of Wnt signaling. *J. Bone Miner. Res.* 22(5), 708–716 (2007).
15. Brown AK, Quinn MA, Karim Z *et al.*: Presence of significant synovitis in rheumatoid arthritis patients with disease-modifying antirheumatic drug-induced clinical remission: evidence from an imaging study may explain structural progression. *Arthritis Rheum.* 54(12), 3761–3773 (2006).
16. Pettit AR, Walsh NC, Manning C, Goldring SR, Gravalles EM: RANKL protein is expressed at the pannus-bone interface at sites of articular bone erosion in rheumatoid arthritis. *Rheumatology* 45, 1068–1076 (2006).
17. Diarra D, Stolina M, Polzer K *et al.*: Dickkopf-1 is a master regulator of joint remodelling. *Nat. Med.* 13(2), 156–163 (2007).
18. Horwood NJ, Elliott J, Martin TJ, Gillespie MT: IL-12 alone and in synergy with IL-18 inhibits osteoclast formation *in vitro*. *J. Immunol.* 166, 4915–4921 (2001).
19. Gange CT, Quinn JM, Zhon H, Kartsogiannis V, Gillespie MT: Characterization of sugar binding by osteoclast inhibitory lectin. *J. Biol. Chem.* 279(28), 29043–29049 (2004).
20. Prakken B, Samodal R, Tho D *et al.*: Epitope-specific immunotherapy induces immune deviation of proinflammatory T cells in rheumatoid arthritis. *Proc. Natl Acad. Sci. USA* 101, 4228–4233 (2004).
21. Coleman RE: Bisphosphonates in breast cancer. *Ann. Oncol.* 16(5), 687–695 (2005).
22. Keating GM, Scott LJ: Zoledronic acid: a review of its use in the treatment of Paget's disease of bone. *Drugs* 67(5), 793–804 (2007).
23. Langston AL, Campbell MK, Fraser WD, MacLennan G, Selby P, Ralston SH: Clinical determinants of quality of life in Paget's disease of bone. *Calcif. Tissue Int.* 80(1), 1–9 (2007).
24. Ellies DL, Viviano B, McCarthy J *et al.*: Bone density ligand, sclerostin, directly interacts with LRP5 but not LRP5G171V to modulate Wnt activity. *J. Bone Miner. Res.* 21(11), 1738–1749 (2006).

Affiliation

- Claire E Clarkin, BSc PhD
Department of Veterinary Basic Sciences, The Royal Veterinary College, Royal College Street, London, NW1 0TU, UK
Tel.: +44 207 468 5036;
Fax: +44 207 468 5204;
cclarkin@rvc.ac.uk
- Nicholas C Harvey, MA MB BChir MRCP PhD,
Clinical Lecturer, MRC Epidemiology Resource Centre (University of Southampton), Southampton General Hospital, Southampton, SO16 6YD, UK
Tel.: +44 23 8077 7624;
Fax: +44 23 8070 4021;
nch@mrc.soton.ac.uk