

Advances in the molecular pharmacology and therapeutics of bone disease
and International Symposium on Paget's Disease
July 10–14, 2007
St. Catherine's College, Oxford, UK

This meeting, the third in a biennial series organised by Professor Graham Russell, Botnar Research Centre, Nuffield Department of Orthopaedics, University of Oxford, brought together 180 academic and industry scientists to discuss current and prospective molecular targets for development of drugs for metabolic bone diseases.

Osteocytes in bone remodelling

Presentations on the osteocyte reflected the many interesting recent discoveries around this, the most abundant of all cells in bone. *Brendon Noble* (Edinburgh) described how osteocytes are ideally situated to respond to local mechanical signals, presumably cytokine-mediated, by instructing other bone cells where to work — something that cannot be done by circulating hormones. The nature of osteocyte-derived signals has been elusive until recently, but sclerostin, the protein product of the *sost* gene, is apparently exclusively derived from the osteocyte in bone, and is a powerful inhibitor of bone formation, regulating development through the osteoblast lineage by inhibiting Wnt signalling. *Scott Simonet* (Thousand Oaks, California) described the *sost*^{-/-} mice, with very high bone mass, analogous with the human inactivating *sost* mutation responsible for von Buchem's Disease, as well as reporting on studies in primates showing the efficacy of an anti-sclerostin antibody as a powerful stimulator of bone formation. A human monoclonal anti-sclerostin is now in Phase I clinical study. The reverse syndrome, of severe bone loss, occurs in transgenic mice overexpressing sclerostin in osteocytes, as reported by *Michaela Kneissel* (Basel), who also described how parathyroid hormone (PTH) and PTHrP rapidly and profoundly decrease sclerostin production by osteoblasts *in vitro* and *in vivo*, raising the real possibility that this might make a significant contribution to the anabolic action of PTH/PTHrP, by removing a constitutively expressed inhibitor. Finally, *Teresita Bellido* (Little Rock, Arkansas) showed how osteocytes communicate by gap junction channels, required for anti-apoptotic effects of e.g. PTH and estradiol, and dependent upon expression of connexin 43 (CX43).

The osteoblast as a hormone—control of metabolism

Patricia Ducy (New York) revealed a remarkable role for bone as an endocrine organ, using genetically manipulated mice to discover that osteocalcin promotes increase insulin production by pancreatic β -cells, increased insulin sensitivity in fat and muscle, and decreased visceral fat. Osteocalcin-deficient mice are mildly diabetic and obese. Mice null for a protein tyrosine phosphatase product of the *Esp* gene were hypoglycaemic, with increased pancreatic islet size and β -cell number, increased insulin sensitivity and decreased body fat. This phenotype was identical in global knockout and in osteoblast-specific *Esp* knockout mice, neither of which showed any detectable skeletal phenotype. The metabolic phenotype was fully rescued by crossing the *Esp* null mice with *osteocalcin*^{+/-} mice. Furthermore *in vitro* experiments established that osteocalcin enhanced insulin production by islets, and increased insulin sensitivity. Most intriguingly, the true hormone appears to be osteocalcin that is not γ -carboxylated. The concept of the skeleton as a ductless gland, responding to environmental influences upon metabolism and energy requirements by producing a hormone with such actions, is a revolutionary one.

Approaches to skeletal anabolic therapies

Roland Baron (New Haven, Connecticut), identified multiple levels of intervention with the Wnt pathway as a bone anabolic target—receptor interaction (including sclerostin), DKK-1, and the GSK enzyme. DKK-1, produced predominantly in osteoblasts and osteocytes and inhibiting Wnt signalling through its binding to LRP5, is an attractive target, and some preclinical studies show promising effects of neutralising antibody. Preclinically also, GSK inhibition has resulted in increased bone formation. One of the concerns has been that the link between the Wnt pathway and cancer formation might provide risk, but this has not yet emerged in preclinical studies. *David Dempster* (New York) reviewed the only established anabolic, PTH, which promotes osteoblast differentiation of committed precursors and inhibits osteoblast apoptosis. The latter is based on data from

rodent studies, but Dempster detected no such effect in studies of biopsy material from PTH-treated human subjects.

Some antiresorptives including SERMs

Other direct therapeutic approaches that were considered in addition to reviews of clinical efficacy of BPs were strontium ranelate and vitamin D analogs. *Rene Rizzoli* (Geneva) reviewed the clinical success in fracture prevention with Sr ranelate, which appears to act as a resorption inhibitor, without any inhibitory effect on bone formation, as assessed by bone marker assays. *Roger Bouillon* (Leuven) noted the importance of using the skeletal anabolic action that can be shown in vitamin D analogs, but developing analogs that separate this effect from the undesirable hypercalcemia. He introduced the concept of the vitamin D receptor modulator, which was extended by *Henry Bryant* (Indianapolis), who reported promising studies showing a substantial improvement in separation of the anabolic from the hypercalcemic/hypercalciuric effect. *Bryant* also discussed selective estrogen receptor modulators (SERMs), stressing the importance of their effects on receptor shape change and interaction with co-regulatory proteins, and the need for structure–activity relationship (SAR) that diverges from the natural ligand. The SERMs in current trials appear more potent than those existing, are more biologically available, uterine safety seems improved, the anti-breast cancer effect maintained, and in some cases there is potential for less vasomotor side effects. Finally, among the anti-resorptive class, *Nathalie Franchimont* (Zug, Switzerland) outlined the impressive efficacy of monoclonal anti-RANKL in preclinical studies, including primates, as well as in a completed phase II study that showed the remarkably prolonged effect of the antibody (denosumab) in suppressing bone resorption parameters.

Osteoimmunology and the bisphosphonate connection

Matthew Gillespie (Melbourne, Australia) discussed the contribution of lymphocyte-derived cytokines to the growth and development of bone cells. T cell products favouring osteoclast formation by different mechanisms include IL-6, IL-7 and IL-17, and several pro- and anti-inflammatory T-cell cytokines act either directly or indirectly to inhibit the process, potentially contributing to the bone destruction of inflammatory arthritis and periodontal disease. Interestingly though, increased osteoclast formation *in vivo* in mice rendered null for IL-12, IL-18 and the compound null mice, suggested a physiological role for these cytokines. The immune system was linked with bisphosphonates used in therapy, where the nitrogen-containing bisphosphonates (N-BPs) interact with a T-cell subset to explain the acute phase reaction, which commonly accompanies their use. *Andrew Sewell* (Cardiff) showed how $\gamma\delta$ T cells are induced rapidly by N-BPs to produce excessive TNF and IL-6, an effect prevented by incubating the cells with statin drugs, that are mevalonate pathway inhibitors — implying their possible use in preventing the acute phase reaction. Among interesting questions are whether the $\gamma\delta$ T cell effect might play a role in other possible therapeutic benefits of N-BPs. These include their

anticancer properties, for which there is some supportive *in vitro* evidence, and actions against certain parasitic and bacterial infections, particularly in the intestine.

Help for the field from new technologies

A session on advanced technologies and imaging was a highlight of the meeting. *Udo Opperman* (Oxford) gave an account of activities of a structural genomics and drug discovery consortium that has solved over 400 protein structures in its first 4 years. The structural analysis of farnesyl pyrophosphate synthase (FPPS) complexed with N-BPs has shown the drugs binding in either of two isoprenoid pockets, and sidechains positioned so that the phosphate groups bind to Mg ions. New insights into structure activity relationships are resulting from these analyses, and are being applied to the development of BPs targeting parasitic diseases, for example attacking the isoprenoid pathway in the protozoan, *cryptosporidium parvum*. Many of the implications of these structural insights were discussed in depth by *Michael Rogers* (Aberdeen) in an update of how the cellular actions of BPs are mediated. Nano-computer tomography and the synchrotron were methods used by *Ralph Muller* (Zurich) in describing exquisite detail of bone structure, particularly showing the enormous abundance of osteocytes — as many as 60,000/c.mm — and their communicating canals, and allowing visualisation of osteocyte lacunar volumes, that vary with size of mouse bone. Osteocytes were concluded not to play a major role in initiating microcracks, but the pattern of microcracks could be shown by nano-CT, developing internally at blood vessels and the pattern associating closely with blood vessels in the cortex. In discussing applications of genetics to human disease, especially osteoporosis, *Matthew Brown* (Brisbane, Australia) pointed out how linkage studies in the absence of monogenic effect have not been helpful, and that few genes can be regarded as having “established” relationships with bone mineral density. He argues that this will change, proposing that genome-wide association studies with linkage disequilibrium mapping is the way forward. Progress has been dramatic in the last 5 years, with reduced genotyping costs, improved accuracy and statistical analysis, the number of SNPs required reduced, and case-control or related cohort designs are now possible. From the HapMap Consortium (Caucasian, Chinese, Japanese and Nigerian populations) SNPs are freely available. With such approaches 25 definite and 50 very likely genes of importance in diabetes have been identified (Nature 447:661, 2007; data available at: www.wtccc.org.uk). In describing approaches to identifying new markers for bone, *Jude Onyia* (Indianapolis) used genomic and proteomic analyses applied to studies of rodent bone in parallel with measures of bone strength, with antiresorptive or anabolic treatments and following estrogen withdrawal. These revealed gene expression signature patterns that can predict responsiveness to drugs in the preclinical models. Notable are the relationships among osteogenic, chondrogenic and adipogenic markers, for example with cartilage and adipocyte markers increasing after ovariectomy. An interesting aspect of the chondrocyte markers (e.g. collagen II crosslinks) is their consistent appearance in bone — in the metaphysis with careful

exclusion of the growth plate region, but in diaphysis as well, confirmed in several species by mass spectrometry. The search continues for discriminating individual markers, but early success may be more likely with patterns of genes.

Inflammatory arthritis and cancer

Paul Emery (Leeds) summarised the dramatic changes in rheumatoid arthritis, that has gone from an incurable disease to one with many effective treatments. It is only fully reversible at early stages, a diagnosis helped by using anti-CCP as a very specific early marker of disease. Progress of disease and response to therapy can now be assessed using imaging, with MRI able to show correlation between synovitis and erosion of bone. Drugs such as methotrexate can help clinically but rarely with imaging remission. With the availability of anti-TNF biologics, even though these are limited because of expense, 50% of the worst affected patients can achieve remission with anti-TNF plus methotrexate. Alternative biologics such as neutralisers of IL-6 and IL-17 are under consideration, as well as the idea of a combined attack on the inflammatory process together with inhibitors of bone resorption. *Gregory Mundy* (Nashville) recognised the importance of the osteoclast in the invasion of bone by cancer cells, particularly breast and multiple myeloma, but also in prostate cancer, where early in the bone metastasis process osteoclastic resorption appears to be important. This is the basis of the therapeutic efficacy of resorption inhibition in prevention and treatment of bone metastases. He made a strong case also for a key contribution of osteoblastic cells to the metastasis process, citing for example the effect of DKK-1 as a product of myeloma or cancer cells, reducing osteoblast differentiation and bone formation locally and thus contributing further to tumor expansion. These concepts of how the bone microenvironment contribute in very specific ways to cancer invasion have been expanded by new understanding of the “metastatic niche”, where a number of host cells change gene expression upon the arrival of tumor cells and set the scene for tumor cell growth. The role of the tissue microenvironment is a topic of great interest in cancer biology. Of all sites of tumor metastases, bone may provide the most readily recognisable processes to investigate, and targets for the development of drugs to prevent this.

Paget’s Disease of bone

Epidemiology and genetics

The section of the meeting devoted to Paget’s Diseases provided much new information on both pathogenesis and treatment of the disease. *Tim Cundy* (Auckland) drew attention to the accumulating data which shows that both the prevalence and severity of PDB seems to be decreasing in some countries especially New Zealand and the UK which were formerly high incidence areas. Suggested explanations include environmental toxins, diet, mechanical loading of the skeleton, infections and changes in ethnic mix of populations due to migration. The reduction has not been seen in all regions and in particular,

studies from the USA and Italy have shown a stable incidence in PDB over recent decades. *Cundy* presented compelling evidence favouring an environmental influence (at least in New Zealand) however, in showing that the onset of PDB was delayed by about 5–10 years in children of SQSTM1 mutation carriers compared with their parents.

Stuart Ralston (Edinburgh) summarised the importance of genetic factors in PDB and related disorders. Mutations in four genes (RANK, OPG, SQSTM1 and VCP) have now been identified that cause PDB or PDB-like syndromes, and all of these are involved in the RANK-NFκB signaling pathway. Although SQSTM1 mutations are the most important cause of classical PDB, many other genes remained to be discovered and the two hottest candidate loci currently lie on chromosomes 5q31 and 10p13. Many presentations focused on the role of SQSTM1 mutations in PDB. Although most mutations had been identified in exon 8 and directly affect the ubiquitin associated (UBA) domain, there is emerging evidence to suggest that mutations in exon 7 outside the UBA domain can also cause the disease. It is thought that these might possibly causing a conformational change in the UBA and impair its ability to bind to ubiquitin. The emerging theme is that truncating mutations (which delete the UBA domain) cause more severe disease than missense mutations. *Rob Layfield* (Nottingham) presented ways to quantitate Ubiquitin binding affinity of different mutations, which would in the future allow for a more precise definition of the relationship between genotype and phenotype. Subsequent presentations focused on the rare syndromic forms of PDB. *Michael Whyte* (St. Louis) reviewed the syndromes caused by activating mutations of RANK and inactivation mutations of OPG, with evidence that antiresorptive therapy is of benefit to the skeleton in both situations but noting that in Juvenile Paget’s Disease, this might not be effective at preventing the development of cardiovascular mortality. This highlights the importance of OPG not only in bone but also in blood vessels. *Virginia Kimonis* (Irvine, CA) provided insights into the rare syndrome of inclusion body myopathy, Paget’s Disease and frontotemporal dementia (IBMPFD). The main morbidity and mortality in this syndrome is caused by cardiorespiratory failure due to myopathy or dementia, but in many cases the PDB component can also be severe and symptomatic, occurring on average by the age of 40. Electron microscopy of osteoclasts and muscle cells in a patient with IBMPFD were classical of the “viral inclusions” found in classical PDB. This raised much discussion as to whether or not the inclusions are actually misfolded protein aggregates rather than viruses, which was suggested by the work of *Miep Helfrich* (Aberdeen) also presented at the meeting.

Animal models of Paget’s Disease at last

David Roodman (Pittsburgh) presented new data on the SQSTM1 P329L “knock-in” mice. He indicated that osteoclasts and stromal cells from these mice shared many features in common with PDB but to date albeit had not been possible to define a clear *in vivo* phenotype, at least by histological analysis of vertebral bone. In contrast, *Javier Rojas* (Edinburgh) and

colleagues showed that mice with a truncating mutation of SQSTM1 developed a bone disease strikingly similar to PDB with focal osteolytic lesions showing increased osteoclast and osteoblast activity. It was speculated that the differences may be due to the fact that the truncating mutation is more deleterious (and therefore has a higher penetrance), or that differences in background strain of the mice may play a role. *Ian Reid* (Auckland) presented the results of a microarray analysis of gene expression in Pagetic bone marrow cultures and osteoblast cultures. Various differences between PDB bone and control samples were observed including an increase in DKK1 in PDB samples. Reid also used the opportunity to look for somatic mutations of SQSTM1 in these Pagetic cultures (none were found) and to look for measles virus. There was no evidence of measles viral transcripts with a highly sensitive assay that could detect as few as 16 transcripts. Although Reid acknowledged that it was impossible to exclude the presence of viral mRNA completely, a positive control from SSPE was included in the assay and if measles was present it was 400 times less abundant than in SSPE!

Evaluation of treatments

The session on treatment focused on existing therapies and the outcome of new approaches. *Anne Langston* (Edinburgh) presented final results from the PRISM study showing that in this cohort of over 1300 PDB patients with advanced PDB, there was no advantage in giving “intensive” bisphosphonate treatment in terms of any major clinical outcome including fractures. Equivalent results were obtained with “symptomatic”

treatment where NSAID and painkillers were used in combination with occasional courses of bisphosphonates to control bone pain. This indicates that at present symptomatic therapy remains a valid treatment option in patients with established disease. Langston also drew attention to the fact that quality of life scores and alkaline phosphatase (ALP) levels were very poorly correlated in PDB patients, emphasising that clinicians should be “treating the patient and not the alkaline phosphatase”. Although ALP levels do not correlate well with morbidity in advanced disease there is the hope that treatment with potent bisphosphonates in early disease might prevent progression. *David Hosking* (Nottingham) presented results from the HORIZON studies in PDB and showed that if any drug was going to be effective in prevention it was likely to be Zoledronate. In almost 90% of Zoledronate treated patients ALP was normalized, and quality of life scores also improved to an extent. Further long-term follow-up of this cohort looking at hard clinical endpoints and quality of life would be of great interest.

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