MEETING REPORTS

Advances in the Molecular Pharmacology and Therapeutics of Bone Disease and International Symposium on Paget’s Disease

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T. John Martin¹ and Tim Cundy²

¹St Vincent’s Institute of Medical Research, Melbourne, Australia
²University of Auckland, Department of Medicine, Auckland, New Zealand

This meeting, the fourth in a biennial series organized by Professor Graham Russell, Botnar Research Centre, Nuffield Department of Orthopaedics, University of Oxford, was aimed at bringing academic and industry scientists together to discuss current and prospective molecular targets for the development of drugs for metabolic bone diseases. The topic, together with mechanisms and treatment of Paget’s disease, was covered broadly and in depth, with 190 participants at the 4-day meeting (see http://www.molpharm2009.org/)

Intercellular Communication in Bone Remodeling

Much attention in bone biology presentations was directed to mechanisms of intercellular communication in bone (1), from the control of osteoclasts by the osteoblast lineage, to the influence of cytokines from immune cells, and the most recent topic of interest, how osteoclasts might influence osteoblast differentiation and bone formation, and most importantly, how far the influence of osteocytes goes into all aspects of bone biology.

Lynda Bonewald (Kansas City) described the intricate connections between osteocytes (the most abundant cells of bone by far) themselves as well as their connections with surface cells through which they signal to inhibit bone formation (2). Most interesting was data from pregnant mice that showed enlargement of osteocytic lacunae, and frequent staining of osteocytes with tartrate-resistant acid phosphatase – resurrecting the old idea of “osteocytic osteolysis” – and a contribution of the osteocyte network to extracellular calcium. Other intriguing aspects of osteocyte biology were addressed by Michaela Kneissel (Basel). Having shown high bone mass in Sost null mice and decreased bone in sclerostin transgenic mice, and that Mef2 transcription factors control the Sost distal enhancer (3), mediating PTH inhibition, she described mouse models with altered Sost gene dosage showing that sclerostin suppression by intermittent PTH treatment contributes to the anabolic action of PTH. Using double null LRP5 and Sost mice it was found that the relative bone gain in sclerostin deficiency is blunted in the double null mice. Evidence was produced showing that sclerostin targets LRP5 and other pathways in osteoblasts, and that LRP6 production is enhanced in LRP5 deficiency. Most interestingly, LRP4 was discovered as a novel osteocyte-derived binding partner for sclerostin, facilitating sclerostin inhibition of osteoblast function in vitro, and shifting to the left the sclerostin dose response of inhibition of Wnt signaling. Finally, as little as 2.5 weeks’ treatment of aged mice with anti-sclerostin antibody resulted in substantial increases in bone formation. Co-treatment with zoledronate as a resorption inhibitor maintained bone mass.

Another connection with sclerostin came from Natalie Sims (Melbourne), pursuing the role of gp130 signaling in bone, who reported the anabolic action of oncostatin M (OSM) with local injection over calvariae, but also its ability to inhibit sclerostin production as effectively as PTH. Surprisingly, this effect persisted in OSM receptor null mice as the only discernable remaining OSM action. Both OSM and LIF use the gp130
signaling transducer, but whereas OSM stimulation of RANKL production uses the OSM receptor, OSM lowered sclerostin production through the LIF receptor (LIFR), raising the possibility that this OSM action through the LIFR could be an anabolic target without influencing bone resorption.

The role of PTHrP locally in bone was presented by David Goltzman (Montreal), identifying with mutant mice the key role played by local PTHrP in bone remodeling. He also described mice with “knock-in” in osteoblasts of truncated PTHrP, missing the nuclear localizing and C-terminal sequences. These mice had severe premature osteoporosis and impaired cell cycle progression, illustrating the biological importance of PTHrP sequences beyond the N-terminal portion that is recognized by the PTH/PTHrP receptor.

The relationship between the osteoblast and adipocyte lineages is of increasing interest. Clifford Rosen (Portland, Maine) reported that activation of adipocyte differentiation from marrow stromal cells with the drug rosiglitazone resulted in decreased bone in mice in a strain-dependent manner, highlighting a strong genetic background effect. He also reported on the action of nocturnin, a product of the Noc gene, which is implicated in the post-transcriptional regulation of mRNA stability in a circadian manner. Nocturnin acted early (before PPARγ induction) in the adipocyte differentiation pathway to favor adipocyte differentiation.

Insights into bone remodeling mechanisms were provided through the application of gene arrays to bones of normal and ERα(-/-) mice in response to loading (Joanna Price, London) (4). Bone is known to respond very rapidly to changes in loading, but the changes in gene expression in response to only a 30-second load stimulus took place very rapidly, and determined bone formation effects that could only be seen days later as increased numbers of osteoblasts. One of the interesting observations was that in ERα(-/-) mice, the number of genes differentially regulated by loading was markedly reduced at all time points. The expected decrease in SOST expression took place in loaded normal mice but was lost in the ERα null mice, supporting the proposition that there is a role for ERα signaling in the response to loading of bone.

New Regulatory Mechanisms in Bone

In discussing the increasing evidence for neuroendocrine regulation of bone metabolism (5;6), Gerard Karsenty (New York) showed this to be bi-directional. A loop was completed from fat to brain to bone to energy metabolism with a dipocyte-derived leptin acting centrally to use peripheral sympathetic tone to increase the amount of bone. This increased sympathetic activity, acting on the osteoblast through the transcription factor ATF4 also increased the enzyme that appears to favor carboxylation of osteocalcin, thereby reducing the generation of under-carboxylated osteocalcin, the hormonal form that favors insulin secretion and sensitivity and energy utilization. The second major change in thinking came from Patricia Ducy (New York), showing that although LRP5 null mice have reduced osteoblast proliferation and low bone mass, these abnormalities are not present during development or at birth. Together with the observation that osteoblast-specific β-catenin null mice have a resorption phenotype, this led to gene array studies that revealed over-expression in LRP5 null mice of Tph1, the enzyme used in the proximal intestine for the synthesis of serotonin. By using a number of genetically manipulated mice it was then shown that serotonin, produced in enterochromaffin cells of the duodenum, is a powerful hormonal inhibitor of bone formation (7). This raises many interesting questions, including the relative inputs to bone formation regulation of LRP5 functioning in the intestine on the one hand, and on Wnt signaling in bone on the other - all yet to be resolved (see recent BoneKEy Commentary by Roland Baron).

There is no shortage of new pathways that profoundly influence bone metabolism, as exemplified by Aymen Idris (Edinburgh), extending previous work that showed reduced bone mass in mice deficient in type
2 cannabinoid receptors (CB2 KO). These mice attained a normal peak bone mass, but rapidly lost bone by 12 months because of defective bone formation, with ex vivo studies showing cell-autonomous impairment of osteoblast differentiation. CB2 selective agonists were able to enhance osteoblast differentiation in vitro and reverse ovariectomy-induced bone loss.

Lessons From Human Genetics

A number of genetic abnormalities of the skeleton were reviewed by Francis Glorieux (Montreal), particularly collagen mutations that result in the osteogenesis imperfecta syndromes of varying severity. New information raises the possibility that osteoporosis pseudoglioma syndrome might be a disease originating in the upper small intestine, by virtue of the LRPS link with serotonin production (v supra). Much has been learned of parathyroid gland development from genetic mutations involving transcriptional control mechanisms (Raj Thakker, Oxford). Matthew Brown (Brisbane) pointed out the advances being made in understanding of disease causation of complex diseases through genome-wide association studies (GWAS), and this is to be expected in osteoporosis. It has been possible through great technical advances in sequencing technology and its high throughput, but will require much greater effort to be made with functional genomics if it is to lead to treatments, especially since all associated genes for osteoporosis thus far have contributed only a few percent each, at most, to disease.

Hypophosphatasia, due to mutations in the alkaline phosphatase gene, manifests itself in a range from very severely affected infants to mildly affected adults. A great boost to prospects of gene replacement therapy came with the report by Michael Whyte (St. Louis) that an engineered form of alkaline phosphatase, targeted to bone with aspartic residues added, and proprietary chemical modification (Enobia, Montreal) has been successful in treating mice rendered alkaline phosphatase-deficient, but most importantly, shows real promise in the treatment of some very severely affected children. This exciting advance clearly has implications for bone-targeted therapy for other disorders as well.

Therapeutic Targets – Current and Projected

The basic biology of this conference provided a platform for discussing approaches to treatment, exemplified by the rationale of development of anti-RANKL (denosumab) as a resorption inhibitor (Paul Kostenuik, Thousand Oaks), and shown in 16 months’ treatment in monkeys to decrease bone remodeling and increase bone mass and strength, with the lowest levels of bone turnover associated with greatest strength in cortical and trabecular bone, and decreased cortical porosity and intra-cortical bone turnover. The data from a double-blind, placebo-controlled study of 7800 women aged 60-90 years (Nathalie Franchimont, Zug) treated with 6-monthly injections of denosumab showed reduction in hip, vertebral and non-vertebral fractures. These findings present an interesting new player in the management of bone loss. This was discussed from a number of points of view. Erik Eriksen (Oslo) raised the issue of the effect of long-term suppression of bone turnover by such effective approaches as prolonged RANKL suppression and powerful bisphosphonates (BPs). Serge Ferrari (Geneva), in discussing PTH combination with anti-resorptive agents, gave some examples of anti-resorptives increasing the bone mass effect of PTH when given together in rodents and humans, but when intermittent PTH and RANKL blockade were administered together in mice that was no more effective than RANKL blockade alone, suggesting that there may be a role for osteoclasts (actually bone remodeling) in the full expression of the PTH anabolic effect.

What used to be called SERMs are now estrogen agonists/antagonists (EAAs), by decree of the U.S. Food and Drug Administration. The most recent drugs in this class are lasofoxifene and bazedoxifene, both of which have reduced vertebral fractures in controlled studies. Arzoxifene is next in development, currently in a large fracture study, and compares favorably with
raloxifene in phase 2 study with bone marker and bone mineral density evaluations (Richard Eastell, Sheffield). Henry Bryant (Indianapolis) stressed the importance of androgen action in bone, mediating anabolic effects in cortical bone and anti-resorptive effects in trabecular bone through RANKL suppression. Selective androgen receptor modulators (SARMs, yet to get the FDA treatment) are being developed that are anabolic for muscle and for cortical bone. They are not substrates for 5α-reductase or aromatase and as a result, several are under study that have no prostate effects. Ostarine (Merck) is one such compound, which increases both peak muscle tension and 3-point bending strength of bone. Progress with the clinical use of strontium ranelate was reviewed by Eugene McCloskey (Sheffield). This drug is effective in fracture reduction, especially in the elderly, but its mechanism of action in vivo remains elusive.

The calcium-sensing receptor (CaSR) remains an attractive drug target (Edward Nemeth, Toronto), a calcimimetic having already been shown in extensive studies and clinical use to be effective against the secondary hyperparathyroidism of chronic renal failure and its consequences. Efforts continue to arrive at a calcilytic that will transiently promote PTH secretion in such a way that it will be anabolic, without prolonged secretion that thus far has resulted in sufficient resorptive effect such that anti-resorptive treatment has been necessary as an accompaniment. The cathepsin K enzyme is an interesting target, with Sevgi Rodan (Philadelphia) reporting development of Merck’s odanacatib (see recent BoneKEy Perspective by Sevgi Rodan and Le Duong), an effective resorption inhibitor that in preclinical studies in rabbits and monkeys is effective, without inhibition of bone formation. This gives rise to the “new class” or “dual action” resorption inhibitor referred to by Erik Eriksen (Oslo), who asked whether this will improve anti-resorptive treatment. The clinical studies with odanacatib are now underway. Calcitonin is an old target that is being revived through its use in an oral delivery system that is resulting in impressive effects on resorption markers, and is now being applied in a large fracture study (Morten Karsdal, Copenhagen). In addition, because of the calcitonin effect to prevent cartilage degradation, the oral delivery form is being studied in an osteoarthritis trial, with biochemical marker and quantitative MRI endpoints. The anabolic action of prostaglandin E (PGE) on bone has long been spoken of as a drug target, but suffered from the undesirable systemic effects of PGE administration. Vishwas Paralkar (Groton, CT) demonstrated impressive efficacy on fracture healing of intra-lesion injection of synthetic agonists of the EP2 and EP4 forms of the PGE2 receptor.

A novel potential target presented by James Edwards (Nashville) was the silent information regulator (SIRT1) gene, an “aging gene” coding for sirtuin. SIRT1 is a gene silencer expressed much more in young bone than in old, and there is evidence that it is important in favoring adipocyte differentiation from stromal precursors. SIRT1 null mice have decreased bone, with increased osteoclasts and decreased osteoblasts, raising the possibility that SIRT1 activation could both promote formation and inhibit resorption. One such activator is the compound resveratrol (present in red wine), and analogs of resveratrol are in early study for this very purpose.

Fortieth Birthday of the Bisphosphonates

A highlight of the meeting was a celebration of the 40th ‘birthday’ of the BPs.

Despite all the exciting new developments in bone biology and therapeutics, it is a sobering thought that the BPs still currently dominate the market as the most effective agents for the treatment of bone diseases characterized by increased bone resorption. These drugs have a long history and a fascinating odyssey of ‘ups and downs’ in clinical development. This year represents the 40th anniversary of the first reports of the biological effects of the BPs, then called diphosphonates, published by Herbert
The session opened with Graham Russell (Oxford) describing the history of this discovery and the early clinical use of BPs for bone scintigraphy, and the treatment of Paget’s disease. Starting in the 1970s this was extended to hypercalcemia of malignancy, and then to the prevention of skeletal-related events in patients with myeloma or bone metastases. It was not until the 1990s that the BPs became established as drugs for the treatment of osteoporosis, an advance that was enabled by improvements in the technology of bone mineral measurement, which allowed large trials to be done with measurable endpoints. The use of once yearly zoledronate given intravenously represents the latest and impressive advance in optimizing the properties of these drugs for clinical use, for example, in Paget’s disease and osteoporosis (and malignancy). Over the years more than a dozen BPs have been used clinically, and most have been licensed in more than one country for different clinical uses. Several BPs have already or will soon reach the end of their patent lives, and their availability as generics makes them inexpensive and the drugs of choice in this cost-conscious health era, although all of these drugs show such low and variable absorption that they present a significant quality control problem as generic preparations.

By the late 1970s the biological and clinical effects of the first of the nitrogen-BPs, pamidronate, was being reported by Olav Bivoet and his colleagues from Leiden. The Leiden contributions continue to the present day and were described in detail by Socrates Papapoulos (Leiden). Their discoveries included the first descriptions of the immunological effects of BPs in terms of inducing the acute phase response, as well as many contributions to the clinical pharmacology of these compounds.

Hal Ebetino (Cincinnati) described how the anti-resorptive effects on osteoclasts of the nitrogen-containing BPs (including alendronate, risedronate, ibandronate, and zoledronate) appear to result principally from their inhibition of FPPS, a key enzyme in the mevalonate pathway, which generates isoprenoid lipids utilized for the post-translational modification of small GTP-binding proteins that are essential for osteoclast function. Many questions that are being tackled in current research remain unanswered, for example, how do different BPs distribute within bone and enter osteoclasts, and what are the most important signaling pathways that are disrupted in their presence. Mike Rogers (Aberdeen) described how the anti-resorptive effects on osteoclasts of the nitrogen-containing BPs (including alendronate, risedronate, ibandronate, and zoledronate) appear to result principally from their inhibition of FPPS, a key enzyme in the mevalonate pathway, which generates isoprenoid lipids utilized for the post-translational modification of small GTP-binding proteins that are essential for osteoclast function. Many questions that are being tackled in current research remain unanswered, for example, how do different BPs distribute within bone and enter osteoclasts, and what are the most important signaling pathways that are disrupted in their presence.

There are several potential clinical uses of BPs that merit further study, and David Little (Sydney) pointed out several ways in which they could be of value in orthopedics. In
fracture repair they have no significant adverse effects on bone healing, but can retard the remodeling of callus with possible mechanical strengthening at the fracture site. Some of the most intriguing effects are to preserve the structure of joints undergoing bone necrosis (e.g., femoral heads), and this has been used in children and adults with promising clinical outcomes. BPs can also be used to enhance the osseointegration of prosthetic devices.

This interesting session ended with a tea party, and ceremonial cutting of the birthday cake by Marie-Laure Fleisch, daughter of Herbie Fleisch, and Sevgi Rodan.

**Paget’s Disease Symposium**

Simon Mays (Southampton) presented a fascinating review of Paget’s disease in archeological skeletons. He described the numerous pitfalls in making the diagnosis, but applying strict diagnostic criteria, the earliest skeletons known to be affected date from Roman period Britain, which does indeed appear to have been the epicenter of the disease. Tim Cundy (Auckland) discussed the evidence for secular change in the disease, showing that the presentation and severity of Paget’s disease is decreasing across the world (10). This phenomenon was seen even in subjects inheriting sequestosome (SQSTM1) mutations, implying that an environmental factor is crucial to the development of the disease.

The genetics of Paget’s disease continues to be of great interest. Omar Albargh (Edinburgh) presented preliminary results from a genome-wide association study (GWAS) demonstrating strong association with Paget’s disease at loci on chromosomes 1, 10 and 18, and possibly other loci too. Anna Daroszewska (Edinburgh) presented data from two mouse models with knock-in mutations of the two known Paget’s genes, SQSTM1 and valosin-containing protein (VCP). These mice develop Paget-like lesions at the ends of long bones, though these do not recapitulate precisely the human phenotype.

David Roodman (Pittsburgh) discussed a transgenic mouse model expressing p62<sup>P394L</sup> - the murine equivalent of the common SQSTM1 mutation P392L. Bone marrow cultures from these mice formed increased numbers of osteoclasts in response to RANKL, TNF-α or 1α,25-(OH)2D3, similar to Paget’s disease patients. However, purified osteoclast precursors depleted of stromal cells were no longer hyper-responsive to 1α,25-(OH)2D3, whereas co-cultures of purified p62<sup>P394L</sup> stromal cells with either wild-type or p62<sup>P394L</sup> osteoclast precursors formed more osteoclasts than co-cultures containing wild type stromal cells due to increased RANKL production by the mutant stromal cells. Despite the enhanced osteoclastogenic potential of both osteoclast precursors and narrow stromal cells, the p62<sup>P394L</sup> mice had histologically normal bones, suggesting that this Paget’s-associated p62 mutation is not sufficient to induce the disease and that additional factors acting together with p62 mutation are necessary for the development of Paget’s disease in vivo. In other experiments, osteoclasts from mice expressing the measles virus nucleocapsid protein, a factor often implicated but never proven to be implicated in the pathophysiology of Paget’s, did in vitro have many features of the Pagetic phenotype. Clearly there is still much to be revealed about the interaction of genetic susceptibility and environmental factors in the etiology of Paget’s disease.

On the treatment front, the controversy about the indications for BPs rumbles on with differing interpretations of the outcome of the PRISM trial, as discussed by Stuart Ralston (Edinburgh) (11). That study indicates that there is no advantage of intensive BP therapy over symptomatic treatment. However, it is now evident from the long-term follow up of patients treated with intravenous zoledronate (Ian Reid, Auckland) or oral alendronate (Geoff Nicholson, Geelong) that very long remissions can be attained, so that it is now possible to talk in terms of a clinical ‘cure’. As David Hosking (Nottingham) commented in his historical review, this is remarkable progress for a disease for which there was
no effective treatment less than 40 years ago.

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References


