Advances in the molecular pharmacology and therapeutics of bone disease

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This meeting, the second in a biennial series organized by Graham Russell, Botnar Research Centre, Nuffield Department of Orthopaedics, University of Oxford, UK, aimed to bring academic and industry scientists together to discuss current and prospective molecular targets for the development of drugs for metabolic bone diseases and arthritis. The topic was covered broadly and in-depth, with 180 participants over the 2-day meeting.

Osteoporosis, rheumatoid arthritis & osteoarthritis

Although the mechanisms, drugs and potential drugs considered at this conference are obviously relevant to the treatment and prevention of osteoporosis, clinical aspects of the latter were not on the program. Rather, there were two useful reviews of rheumatoid arthritis and osteoarthritis, by Stephen M Krane (Boston, MA, USA) and Tim Spector (London, UK), respectively. In each case, emphasis was placed on the developing realization that increased bone turnover plays an important part in pathogenesis.

Osteoclasts in the coupling of bone formation to resorption

With the advent of anabolic therapies for osteoporosis and their potential interactions with antiresorptive drugs, there is a renewed interest in the process of coupling of bone formation to resorption in the bone multicellular unit. The many components of this process were reviewed by T John Martin (Melbourne, Australia), who described how osteoblast lineage cells can themselves help to regulate the filling of resorption spaces through intercellular communication via growth factors and cytokines. Although it has long been accepted that the resorption of bone is needed to promote bone formation in the remodeling sequence, evidence shows that the active osteoclast itself might be the source of regulatory, bone-forming activity. This osteoclast-derived activity might also contribute to the anabolic effect of parathyroid hormone (PTH), complementing its direct effect on committed preosteoblasts to enhance their differentiation and to inhibit apoptosis of mature osteoblasts and osteocytes. Evidence in support of such osteoclast-derived activity was provided from the interesting work of Morten Karsdal (Nordic Biosciences, Copenhagen, Denmark), in studies of the chloride-7 channel (ClC-7) in osteoclasts. Inactivating mutations in man result in osteopetrosis, as does gene inactivation in the mouse, because the ClC-7-deficient osteoclasts fail to acidify the resorption space and, therefore, virtually lose the ability to resorb bone. Nevertheless, the osteoclasts, both in the human and mouse, look otherwise normal and are present in increased numbers as they are less susceptible to apoptosis. Notably, both in the human genetic mutation and in the knockout mouse, bone formation is normal or increased. Karsdal also reported studies with a low molecular weight inhibitor of the ClC-7, which inhibited bone resorption in the ovariectomized (OVX) rat without inhibiting bone formation. The combined data suggests that the nonresorbing osteoclasts produce a level of activity that favors bone formation. A similar mechanism appears to operate in vacuolar-adenosine triphosphatase (ATPase) genetic ablation and perhaps also in *c-src* ablation. This work raises the possibility of generating new classes of resorption inhibitors that spare the bone formation process.

PTH & other anabolic therapies for bone

The molecular mechanisms of PTH action on bone were addressed by Jude E Onyia (Eli Lilly & Co., Indianapolis, IN, USA), who described gene array studies in delayed-onset OVX rats treated with either intermittent PTH or a glycogen synthase kinase (GSK) inhibitor. The latter was used because of evidence the group had obtained of PTH effects on the wingless-type (Wnt) signaling pathway in bone. With 2400 genes regulated by PTH, 1500 by the GSK inhibitor and 1900 by OVX alone, 159 genes were common in all three states and can therefore be regarded as anabolic markers. The PTH-responsive genes were clustered into those associated with morphogenesis, programmed cell death, growth and differentiation, and with the Wnt signaling pathway. Bone remodeling markers that increased in response to PTH and GSK inhibition were unaffected in rats treated with estrogen or with 1.25 $(OH)_2$ vitamin D or its analogs. Surprisingly, PTH increased expression of genes that are cartilage markers, for example aggrecan, collagen II and X, cartilage (C-type lectin), as well as decreasing peroxisome proliferation-activated receptor (PPAR)-γ, lectin and other adipocyte markers. Thus, the array approach is yielding a wealth of information.

The PTH approach also received attention through Edward Nemeth (NPS Pharmaceuticals, Toronto, Canada) who reported on the development of calcilytic drugs – calcium receptor antagonists that work by substituting for calcium on the Ca receptor of the parathyroid cell, which responds with a burst of PTH secretion. The aim is to develop a drug that has a very short half-life and achieves a rapid and transient peak of PTH release. This was achieved with NPS89636, with the increased PTH level returning to baseine by 2 h. This drug was able to stimulate bone formation in the delayed OVX rat model, and is now in a Phase I clinical study. The opposite approach with calcimimetics – a drug that mimics calcium by inhibiting PTH secretion – is already a success story with cinacalcet (Sensipar®, Amgen), which is in clinical use in primary and secondary hyperparathyroidism, and is indeed the first drug to act allosterically on any G-protein-coupled receptor (GPCR).

Proof of the concept that the Wnt/frizzled signaling pathway provides potential targets for skeletal anabolics came from Georges Rawadi (Prostrakan, Paris, France). This likelihood had been raised by the discovery a few years ago that mutations in LRP-5, a coreceptor for the action of Wnts on the pathway, were associated with a striking bone phenotype. Inactivating mutations resulted in the severe osteoporosis of the osteoporosis pseudoglioma syndromes, whereas a mutation that prevented the action of the inhibitory decoy receptor protein, dickkopf (DKK)-1, resulted in a high bone-mass syndrome. Rawadi established that the Wnt signaling pathway is required for the actions of bone morphogenetic protein (BMP)-2 and sonic hedgehog on differentiation of mesenchymal cells to osteoblasts. Although DKK-$1^{-/-}$ mice die *in utero*, DKK-$1^{+/-}$ mice are viable, with an increased amount of bone, bone formation rate and osteoblast numbers. Using treatment with lithium chloride (a GSK inhibitor) to mimic Wnt signaling treatment of either LRP-$5^{-/-}$ (osteoporotic) or wild-type mice, resulted in increased bone formation. It remains unclear which are the most important Wnt and frizzled molecules in the osteoblast lineage – Wnt 3a and 10b are both strong candidates – but the pathway is an attractive one for drug development. Approaches favored by the direction of this work are Wnt mimetics, and small molecule inhibitors of DKK-1 interaction with LRP-5.

A further approach to anabolic drug development came from Ross Garratt (Osteoscreen, San Antonio, TX, USA), who used the BMP-2 promoter as a target in a high-throughput screen, among the hits of which were the statin class of drugs. Simvastatin was shown to increase bone formation *in vitro*. Very high doses (10 mg/kg) were required for an *in vivo* effect due to the liver metabolism of these drugs and low peripheral delivery. When formulated to be absorbed through the skin and tested over bone or fracture sites, very low doses were effective, particularly with lovastatin. The possibility of local therapeutic uses was raised, particularly regarding dental applications, and Garratt reported promising data using simvastatin coating of implantation devices. Interestingly, the downstream metabolites of the mevalonic acid pathway also inhibit BMP2 promoter activity, a point canvassed at the meeting by Michael Rogers (Aberdeen, Scotland) who also pointed out the powerful effects of statin drugs as inhibitors of bone resorption.

Another interesting product of the BMP-2 promoter screen was a proteasome inhibitor, the effect of which was 10–100-times more potent than that of statins. High proteasome activity is associated with an increased production of gli 3, which inhibits the transcription of several genes. Proteasome inhibition, on the other hand, leads to an increase in the transcriptional enhancers, gli-2 and gli-3, which increases activity of the promoter for BMP-2 and other genes. The statin effects results from different actions but the activity of gli-3 is reduced, probably by an effect on phosphorylation. The proteasome inhibitor increases the amount of bone and bone formation rate in mice. Interestingly, a drug of this class, bortezomib (velcade®, Millenium Pharmaceuticals, Cambridge, MA, USA), is now in use in patients with multiple myeloma, where it appears to be capable of increasing bone formation in addition to blocking myeloma growth.

Prostaglandins are the other known stimulators of bone formation, although best known for the promotion of bone resorption. L Raisz (West Hartford, CT, USA) provided an insight on the information obtained in recent years from studies in mice with genetic inactivation of the receptors, E-2 and EP-4. Much of this was instructive of prostaglandin action on resorption, but both receptors were found to be capable of mediating bone formation effects through cyclic AMP as a signaling molecule. An EP-2 agonist, shown in preclinical work to increase bone formation and fracture healing, is in clinical trials (Pfizer).

New drugs in osteoporosis

An interesting new drug for the treatment of osteoporosis was discussed by René Rizzoli (Geneva, Switzerland). Strontium ranelate (Protelos® Servier Inc., Paris, France) achieved a 52% reduction in clinical fractures within the first year of treatment in a large, double-blind, placebo-controlled trial that has led to registration in the UK, Europe and Australia. Although much needs to be done to understand its mechanism of action, strontium does seem to inhibit bone resorption, perhaps also without inhibiting bone formation, and appears to have a commendable margin of safety in clinical use while achieving satisfactory fracture reduction.

The pure resorption inhibition achievable by blocking the receptor activator of nuclear factor κB ligand (RANKL)/RANK pathway was reviewed by Lorraine Fitzpatrick (Amgen, Thousand Oaks, CA, USA). The decoy receptor, osteoprotegerin (OPG), was very effective in preventing bone loss in animal models of these disorders, including osteoporosis, bone metastases and the subchondral bone loss of experimental arthritis. Although OPG is not currently proceeding to clinical trial, interest centers on the clinical study of a fully human monoclonal antibody (AMG-162) against RANKL (Amgen).

Nuclear receptors, estrogen, selective estrogen receptor modulators & vitamin D

A recent success story in osteoporosis therapy has been the development of the selective estrogen receptor modulator (SERM) class of drug. Relevant to this, Donald McDonnell (Durham, NC, USA) reviewed estrogen receptor (ER) molecular pharmacology, showing that estrogen binding to the ER induces a shape change that leads to the recruitment of a large number of coactivator proteins, the collection of which determines the transcriptional response. The first SERM was tamoxifen, which induced subtly different shape changes in the ER, thereby changing the pattern of coactivator protein recruitment and thus the transcriptional effects. McDonnell provided experimental proof of this concept by using nongenomic combinatorial phage libraries (>10^8 random peptides) to demonstrate how estrogen and various SERMs differ in their conformational effects on the ER. Raloxifene (Lilly) is in current clinical use as a SERM, with tissue-specific effects that allow it to be protective against breast cancer and have no significant agonist effect on the uterus. SERMs in development, lasofoxifene and bazedoxifene, appear to have tissue selectivity profiles similar to that of raloxifene.

Exciting developments for the future will require determination of which coactivator proteins are involved in particular biological responses in various tissues. Compounds might then be developed that will change the ER conformation in ways that will provide the most favorable response for the pathway in these tissues. Henry Bryant (Eli Lilly & Co.,) extended this concept by discussions on what the future holds for SERMs. None of the existing SERMs, whether they be triphenylethylenes (tamoxifen), benzothiophenes (raloxifene) or tetrahydronaphhalones (lasofoxifene), are helpful for the vasomotor symptoms of estrogen deficiency. In some cases (raloxifene as an example), this might be due to insufficient amounts of drug reaching the brain, so that structural modifications might be needed. Alternatively, new experimental models that allow investigation of the mechanism of hot flushes might be helpful, together with the chemistry in arriving at SERMs that can specifically prevent or improve vasomotor symptoms. Other potential advances include the development of selective ER-β agonists, one of which was described with 200-fold greater activity on ER-β than ER-α, which has no effect on uterus or bone but is of benefit in arthritis and inflammatory bowel disease models. Gary Krishnan (Eli Lilly & Co.,) also showed interesting data on a possible selective androgen receptor modulator that was very effective in promoting increases in muscle mass, while having no detectable effect on prostate weight or the uterus. This raises the intriguing possibility of drug development to enhance muscle mass and function, but free of unwanted androgen actions.

The tissue selectivity of nuclear receptor action was extended to vitamin D with the work of Roger Bouillon (Leuven, Belgium), who pointed to the many different effects of vitamin D in several tissues – skin, hair, muscle, brain, bone, immune system and intestine. Actions of several vitamin D analogs were reviewed, highlighting variations in their effects on bone and on the immune system.

Bisphosphonates: new insights into molecular mechanisms

Bisphosphonates were the topic of several lectures, reflecting the many recent advances in this area, both in their mechanism of action and clinical applications. Michael Rogers described how the first-generation bisphosphonates, clodronate and etidronate, get into osteoclasts and form toxic metabolites of ATP that lead to osteoclast apoptosis. The nitrogen-containing compounds, on the other hand, inhibit farnesyl diphosphate (FPP) synthase, the enzyme in the mevalonic acid pathway essential for production of FPP, and the next metabolite, geranylgeranyl diphosphate (GGPP). These are essential for the prenylation of guanosine triphosphatases (GTPases), a process required for osteoclast formation and survival. It was pointed out that this effect of the *N*-containing bisphosphonates would be expected with any cell that allows the drug access. In fact, the osteoclast is the most available target for bisphosphonates. Subtle changes in the sidechain structure of the *N*-containing bisphosphonates can result in substantial changes in potency.

The statin drugs, acting earlier in the mevalonic acid pathway by inhibiting 3-hydroxy-3-methylglutaryl (HMG) coenzyme A (CoA) reductase, also inhibit prenylation and are very potent inhibitors of bone resorption *in vitro*. This effect was discussed in relation to the bone formation effects of these drugs. Later in the mevalonate pathway, prenylation can be blocked by inhibitors of farnesyl transferase, which have no effect on osteoclasts, but blockade of geranylgeraniol transferase (GGTase) I increases osteoclast apoptosis. This theme was taken further by Frank H Ebetino (Procter & Gamble Pharmaceuticals, Cincinnati, OH, USA), who described new developments in the chemical modification of bisphosphonates. This included the replacement of one phosphate with a carboxyl group in risedronate (in compound NE 10790), to create an analog that has very low bone affinity but inhibits the invasive capacity of cancer cells *in vitro*, and shows sufficient antitumor efficacy *in vivo* to encourage further work along these lines. NE 10790 is a selective inhibitor of GGTase 2 rather than FPP synthase.

Ebetino also described experiments from the laboratory of Graham Russell, in which a hydroxyapatite column was used to bind and elute bisphosphonates. This approach revealed significant and reproducible differences in binding affinity, with zoledronic acid showing the greatest affinity. The relative affinities, combined with potency on inhibition of FPP synthase, correlated well with the overall

relative efficacies of the bisphosphonates in inhibiting bone resorption. Said Sebti (Tampa, FL, USA) has focussed on drugs that inhibit prenylation. To do this, he used the molecular steps in prenylation to find farnesyl transferase inhibitors highly selective over GGTase inhibitors, and vice versa. His emphasis is on the regulation of the cell cycle and shows that GGTase inhibition in any cell will lead to G1 block and increased apoptosis. Using this approach, GGT inhibitors that block RhoA and RhoC function are able to promote apoptosis in tumors and inhibit their growth. One such drug, GGTI 24/8, will be in a cancer Phase I study within a year. The possibility of such a drug being effective in bone disease was discussed. It would probably need to be targeted by chemical modification to look like a bisphosphonate.

Finally, a highlight of the meeting came from Udo Oppermann and Kathryn Kavanagh (Structural Genomics Consortium, Oxford, UK) who reported their recently determined structure of risedronate bound to human FPP synthase. Analyzed by protein crystallography with a 2 Å resolution, the protein was revealed as a dimer, with 1 mol risedronate binding to 1 mol FPP synthase at the geranyl PP binding site with 160 nM affinity and dependent on Mg^{2+}. A large central cavity was the site of binding of risedronate and two phosphate groups (derived from the crystallization buffer) that mimic isopentenyl PP binding. A hypothesis arising from their work states that compounds that can place the N in equivalent positions to that shown with the risedronate structure, will constitute active compounds. This approach will provide a template for structure-assisted drug design. This structure, and others from the Structural Genomics Consortium, can be seen at [101,102].

Conclusion

This conference was rewarding for its focus upon a comprehensive range of molecular targets and pathways for drug development, particularly as applied to osteoporosis, but also relevant to arthritis, both rheumatoid and osteoarthritis. Some targets were at a very early stage of investigation, a feature that made the meeting all the more attractive. Others, such as PTH and SERMs, are early in their clinical application, but much is still to be learned of their mechanisms. The success of the meeting owes much to the fact that the organizers achieved such an effective mix of high-quality pharmaceutical industry scientists with their academic counterparts – it made for very productive discussions.

Executive summary

- Communication between osteoclasts and osteoblasts is an essential part of the bone remodeling process.
- The first anabolic therapy for osteoporosis is parathyroid hormone, which activates clusters of genes associated with morphogenesis, growth and differentiation, the wingless-type (Wnt) signaling pathway and programmed cell death.
- Several new anabolic targets are being addressed, including the Wnt signalling and mevalonate pathways, proteasome inhibition and prostaglandins.
- Nuclear receptors provide inhibitors of bone resorption through the estrogen receptor, effects on resorption and formation through the vitamin D receptor and on muscle growth through the androgen receptor.
- Molecular analysis of bisphosphonate action has uncovered the importance of osteoclast survival on the control of prenylation of small guanosine triphosphatases. These drugs target specific enzymes in the mevalonate pathway, opening the way to the chemical synthesis of new drugs that are active in cells other than osteoclasts (e.g., cancer cells).
- Structural biology has revealed interactions at the atomic level between bisphosphonates and human farnesyl diphosphate synthase, opening the possibility of rational drug design.

Websites

101. Structural Genomics Consortium
www.sgc.ox.ac.uk
102. Molsoft: molecules *in silico*
www.molsoft.com

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