



SESSION TIME: 1600 - 1805, Wednesday 25 Oct 2006

Invited Plenary Abstracts

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P18

Pathophysiology and prevention of arthritic bone destruction

[Evange Romas](#)

The University of Melbourne, St. Vincent's Hospital, 41 Victoria Parade, Fitzroy 3065, AUSTRALIA

Bone erosions are a prognostic turning point in rheumatoid arthritis (RA) as they correlate with increased disease severity, progressive functional disability and greater mortality. Focal bone erosions are highly associated with systemic osteoporosis, as both phenomena reflect high inflammatory disease activity.

Key molecular discoveries coupled with elegant animal studies have highlighted a pathogenic role of osteoclasts in bone destruction and the concept that osteoclasts (OCLs) drive focal bone erosions is now widely accepted. OCLs are consistently detected at erosion sites in all animal models of destructive arthritis and RA. Targeted removal of osteoclasts either by TNF-blockade or RANKL-antagonism, or genetic manipulation in arthritis models blocks this bone destruction.

The transcriptional program controlling osteoclastogenesis depends both on RANK and immunoreceptor tyrosine-based activation motif (ITAM) signals in osteoclast precursors. The inflamed synovium generates very high levels of TNF- α which fuels osteoclastogenesis in the context of RANKL through multiple mechanisms, as well as cytokines such as IL-6 and IL-17, which increase stromal cell RANKL expression and/ or reduce OPG production.

Although soluble RANKL is produced by T cells, expression of membrane-bound RANKL by fibroblast-like synoviocytes or osteoblasts (induced by IL-6 or IL-17) may be quantitatively more important for osteoclastogenesis in the inflamed joint. Osteoclast formation in animal models is a swift and dynamic process leading to rapid attack on juxta-articular bone, a prerequisite for early onset structural damage. Cell-to-cell contact between stromal cells and osteoclast precursors efficiently presents key molecular signals (ie: RANKL + M-CSF) for osteoclast differentiation and survival. At the pannus-bone interface in arthritis, the main stromal support cells are probably fibroblast-like synoviocytes, whereas the major support cells in subchondral bone are osteoblasts.

The importance of osteoclast-mediated injury in inflammatory arthritis has prompted renewed interest in bisphosphonates for bone protection. Targeting osteoclasts with bisphosphonates such as zoledronic acid confers significant bone protection in autoimmune or TNF-dependent models of RA. Nevertheless, the clinical utility of bisphosphonates for structural joint protection in RA has yet to be shown conclusively.

In summary, down-regulation of osteoclastogenesis has emerged as a powerful strategy for prevention of arthritic bone destruction. Of note, the bone protection afforded by reducing osteoclast numbers is not conditional on reducing synovial inflammation. Osteoclast production in arthritis may be intercepted at several levels. Enthusiasm for bisphosphonates is tempered by their long skeletal half-life and possible association with osteonecrosis of the jaw. The potent effect of Denosumab on osteoclastic bone resorption has been witnessed in osteoporosis trials and human monoclonal antibodies are likely to become the treatment of choice for blocking RANKL in arthritis.

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Is calcitonin a novel treatment for osteoarthritis?

Karsdal, M.A. and Claus Christiansen

Nordic Bioscience Diagnostics, Herlev, Denmark

Center for Clinical and Basic Research (CCBR), Ballerup, Denmark

Objective: Osteoarthritis (OA) is the most common form of degenerative joint diseases and a major cause of disability and impaired quality of life in the elderly. Experimental and clinical observations suggest that the structural integrity of articular cartilage is dependent on normal subchondral bone turnover, intact chondrocyte function and ordinary biomechanical stresses. Because there is a strong inter-relationship between the subchondral bone and the articular cartilage, an ideal therapeutic agent, in the face of normal biomechanical stresses, might logically be directed at regulating the metabolic activity of both bone and cartilage.

Calcitonin has well-established effects on bone resorption. We have investigated whether calcitonin has direct effect on articular cartilage chondrocytes *ex vivo*, and *in vivo* investigated whether a novel oral formulation of calcitonin would improve cartilage health in a non-traumatic model of OA.

Methods: The localisation and expression of the calcitonin receptor in articular chondrocytes was investigated by immunohistochemistry and RT-PCR. Potential direct effects was tested in the articular cartilage explants model, where cartilage degradation was induced by cytokine stimulation of TNF- α [20ng/ml] + oncostatin M (OSM) [10ng/ml], and cultured with salmon or human calcitonin simultaneously [0.0001-1 μ M]. The changes in cartilage degradation were investigated in the conditioned medium by quantification of C-terminal telopeptides of collagen type II (CTX-II). The effect of calcitonin was investigated *in vivo* using the OVX rats. Rats were administered with an oral dose of calcitonin (2 mg/kg) bound to the carrier 5-CNAC (150 mg/kg) once daily for 9 weeks. Collagen type II degradation was quantified in serum by measuring the released CTX-II. Cartilage erosion in knee joints was evaluated by histology. .

Results: The calcitonin receptor was identified on articular chondrocytes, both mRNA and protein forms. Culturing articular cartilage explants in the presence of TNF- α and OSM resulted in a marked, 100-fold increase in CTX-II release compared to vehicle-treated controls ($p < 0.001$). Addition of salmon calcitonin to the explants culture [0.0001-1 μ M] in the concomitant presence of TNF- α and OSM resulted in a significant and dose dependent inhibition of CTX II release ($p < 0.01$). At 100 nM and 1 μ M of calcitonin treatment in the presence of OSM and TNF- α , calcitonin very nearly abrogated collagen type II release, at day 13,16 and 19 of culture. Proteolytic activity was investigated by zymography. TNF- α and OSM resulted in a strong up regulation of MMP-9 activity and expression. This increase in MMP activity was strongly attenuated by calcitonin at 100 nM and 1 μ M, and the positive control GM6001 [25 μ M], a the general MMP inhibitor, Under *in vivo* conditions, oral treatment with calcitonin induced a 95% decrease in serum CTX-II levels, i.e. chondrocyte-mediated cartilage degradation, and restored cartilage degradation in OVX animals to below sham operated animals.

Conclusions: These results suggest that 1) calcitonin act directly on articular chondrocytes; 2) calcitonin inhibits MMP expression and the related collagen type II degradation, thereby providing direct chondroprotective effects; 3) calcitonin carries potentials for becoming a useful therapeutical option for patients with degenerative joint diseases.

P20**Corticosteroids and bone – mechanisms, treatment**Cyrus Cooper*Professor of Rheumatology and Director, MRC Epidemiology Resource Centre, University of Southampton, UK.*

Five decades have elapsed since Hench documented the efficacy of glucocorticoids in the treatment of rheumatoid arthritis. A few years after the introduction of cortisone as an anti-inflammatory drug, the increased incidence of vertebral fractures among patients became evident, and glucocorticoid-induced osteoporosis was described. Although attitudes towards the use of glucocorticoids in clinical practice have changed in recent years, the agents remain widely used; one recent study reported that over 250,000 patients in the United Kingdom were taking continuous oral glucocorticoids, of which only 14% were receiving any treatment to prevent bone loss.

There are many ways in which glucocorticoids may exert their actions on the skeleton and related tissues. Their overall effects depend on a number of factors including the dose, duration, steroid type and species tested. Glucocorticoid receptors are present in most cell types, including bone cells, and glucocorticoid response elements are present in many genes. In addition, glucocorticoid effects may be mediated via the transcription AP1 and overall there are several hundred genes that can respond to glucocorticoids either directly via GREs or indirectly via AP1. Responses to glucocorticoids can also occur by non-genomic mechanisms, involving the glucocorticoid receptor or mediated via the steroids directly. The most important effect of glucocorticoids is the suppression of bone formation through a variety of mechanisms: (a) Reduced differentiation and activity of cell types in the osteoblast lineage; (b) Modulation of transcription of many of the genes responsible for the synthesis of matrix constituents such as type 1 collagen and osteocalcin; (c) Altered synthesis of many locally acting cytokines which affect osteoblasts (IL-1, IL-6), and growth factors (IGF-1, IGF-2, IGFBP-3, BP-4 and BP-5). The latter effects may contribute in particular to the stunting of growth and retarded skeletal development in children treated with glucocorticoids; and (d) Altered lifespan of osteoblasts and osteocytes through enhanced apoptosis. In addition to these direct effects on bone cells, other mechanisms may also contribute to bone loss. Thus, reduced intestinal calcium resorption and increased renal calcium excretion have been reported after the administration of oral glucocorticoids. Low serum testosterone levels have also been reported in glucocorticoid treated men. Histomorphometric analysis of biopsies from glucocorticoid treated individuals have demonstrated a reduction in bone formation at the cellular and tissue level, resulting in reduced bone volume and trabecular thickness. Higher doses of glucocorticoids, however, are also associated with an increase in bone resorption leading to greater bone loss and disruption of cancellous bone architecture. Individual variability to glucocorticoids is well recognised, but the mechanisms involved have not been established.

The most detailed analysis of the relationship between oral glucocorticoid use and fracture risk was performed in the General Practice Research Database of the UK. In this retrospective cohort study comparing 244,235 oral glucocorticoid users and an equal number of age- and sex-matched controls, the relative risk of any non-vertebral fracture during oral glucocorticoid treatment was 1.33 (95% confidence interval (CI) 1.29-1.38), that of hip fracture was 1.61 (CI 1.47-1.76), that of forearm fracture was 1.09 (CI 1.01-1.17), and that of vertebral fracture was 2.60 (CI 2.31-2.92). A dose dependence of fracture risk was observed. With a standardised daily dose of less than 2.5 mg prednisolone, hip fracture risk was 0.99 (CI 0.82-1.20) relative to control, rising to 2.27 (CI 1.94-2.66) at doses of 7.5 mg or greater. For vertebral fracture, the relative rates were 1.55 (CI 1.20-2.01), rising to 5.18 (CI 4.25-6.31), at these two doses. At the intermediate dose of 2.5-7.5 mg daily, the adjusted relative risk of hip and vertebral fracture was 1.77 (CI 1.55-2.02) and 2.59 (CI 2.16-3.10) respectively. Fracture risk increased rapidly after the onset of oral glucocorticoid treatment, and risk declined towards baseline rapidly after cessation of therapy. The use of bone active medication was extremely low among oral glucocorticoid users (5% used hormone replacement therapy and 1.8% use bisphosphonates during the period of follow-up). These epidemiological data suggest that the current population at risk of developing glucocorticoid-induced fractures in the UK might be as large as 350,000 individuals, and that the vast majority of glucocorticoid-treated individuals have not been evaluated for osteoporosis risk, or commenced on treatment to prevent accelerated bone loss and future osteoporosis fracture.

Loss of BMD associated with oral glucocorticoid administration is greatest in the first few months of glucocorticoid use; however, glucocorticoids contribute to the increase in fracture risk over and above the

effect of low BMD. Thus, for a given BMD, the risk of fracture is higher in glucocorticoid-induced osteoporosis than in postmenopausal osteoporosis. Measurement of BMD using DXA is currently recommended for assessment of fracture risk individuals treated with glucocorticoids below age 65 years who have not sustained a previous fragility fracture. Above this age, or among men and women who have sustained a previous fragility fracture, concomitant osteoporosis medication is justifiable, irrespective of BMD measurement. Evidence for the efficacy of agents in the prevention and treatment of glucocorticoid osteoporosis varies, but beneficial effects on BMD in the spine and hip have been demonstrated for several interventions (alendronate, alpha-calcidol, calcitonin, calcitriol, calcium and vitamin D, clodronate, cyclical etidronate, HRT, pamidronate, teriparatide, and risedronate). Fracture has not been a primary endpoint of any studies of prevention or treatment of glucocorticoid-induced osteoporosis. Nevertheless, a reduction in vertebral fracture has been observed in posthoc or safety analyses of trials of etidronate, alendronate and risedronate. Current guidelines for the prevention and treatment of glucocorticoid-induced osteoporosis suggest that individuals at the highest risk should be advised to commence bone protective therapy at the time of starting glucocorticoids; for example, those aged 65 years or over and those with a prior fragility fracture. In other subjects receiving oral prednisolone, in whom it is intended to continue therapy for at least three months, bone densitometry should be considered. A T-score of -1.5 or lower may indicate the need for intervention with a bone sparing agent, although the effect of age on fracture probability in an individual should be taken into account when making treatment decisions.

Further reading:

Royal College of Physicians of the United Kingdom: Glucocorticoid-induced osteoporosis – Guidelines for Prevention and Treatment. RCP, UK, London, December 2002.

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Myeloma-bone interaction: a vicious cycle

T. Matsumoto and M. Abe

Department of Medicine and Bioregulatory Sciences, University of Tokushima Graduate School of Medical Sciences, Tokushima 770-8503, Japan

Multiple myeloma develops and expands almost exclusively in the bone marrow, and generates devastating bone destruction. Myeloma cells stimulate bone resorption by enhancing osteoclast (OC) formation and suppress bone formation by inhibiting osteoblast (OB) differentiation, leading to destructive bone lesions. In these lesions, OCs and immature OBs create a microenvironment suitable for myeloma cell growth and survival, which can be called as "myeloma niche". Osteoclastic bone resorption is enhanced mainly through the secretion of an osteoclastogenic C-C chemokine, macrophage inflammatory protein (MIP)-1, and MIP-1 acts via its receptor, CCR5, on stromal cells to stimulate RANKL-RANK signaling. OCs thus formed enhance the growth and survival of myeloma cells. B-cell-activating factor (BAFF) and a proliferation-inducing ligand (APRIL) are found to play important roles in the interaction between myeloma cells and osteoclasts for the enhancement of myeloma cell growth and survival. Myeloma cells also suppress bone formation through the secretion of Wnt antagonists, including secreted frizzled-related protein (sFRP)-2 and dickkopf1 (DKK1). However, in the presence of a TGF-beta type I receptor kinase inhibitor (SB431542), the inhibition by myeloma cells of BMP-2-induced OB differentiation is reversed. Because SB431542 does not enhance TCF/LEF reporter activity, down-regulated canonical Wnt signaling by myeloma cells is not affected by an inhibition of TGF-beta signaling. Furthermore, when myeloma cells are co-cultured with terminally differentiated OBs with mineralized nodules, myeloma cell proliferation is markedly suppressed. Thus, enhancement of OB maturation can inhibit myeloma growth and survival. These observations suggest that enhancement of OB differentiation can disrupt myeloma niche and ameliorate destructive bone lesions, and that blockade of TGF-beta actions in the bone marrow microenvironment may become a novel therapeutic approach against multiple myeloma.

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Unique biological function of fibroblast growth factor (FGF) 23Seiji Fukumoto*University of Tokyo Hospital, Tokyo, Japan*

FGF23 was identified as a humoral factor involved in the development of several hypophosphatemic diseases including tumor-induced rickets/osteomalacia and autosomal dominant hypophosphatemic rickets/osteomalacia. FGF23 is the last member of FGF family and belongs to FGF19 subfamily. FGF23 decreases serum phosphate level by suppressing expression of type 2a and 2c sodium-phosphate co-transporter in brush border membrane of renal proximal tubules and lowers serum 1,25-dihydroxyvitamin D [$1,25(\text{OH})_2\text{D}$] by modulating expression levels of enzymes for vitamin D metabolism. Because *FGF23* null mice show hyperphosphatemia and high serum $1,25(\text{OH})_2\text{D}$ levels, FGF23 seems to be a physiological regulator of serum phosphate and $1,25(\text{OH})_2\text{D}$. Mutations in *FGF23* gene which enhances FGF23 activity cause autosomal dominant hypophosphatemic rickets/osteomalacia. In contrast, several missense mutations which enhance proteolytic processing of FGF23 protein and abolish biological activity of FGF23 have been found in patients with familial tumoral calcinosis characterized by hyperphosphatemia. Results so far reported indicate that FGF23 is produced by bone cells and works only in kidney. This means that there should be a specific receptor for FGF23 in kidney. Recent in vivo and in vitro investigations identified klotho as an integral molecule for FGF23 signaling. Klotho mice with disruption of *klotho* gene show high serum phosphate and $1,25(\text{OH})_2\text{D}$ levels as *FGF23* null mice. FGF23 level in klotho mice is extremely high indicating that FGF23 can not work in these mice. In addition, introduction of klotho into several cell lines in vitro enables these cells to respond to FGF23. Furthermore, FGF receptor 1 together with klotho and FGF23 forms a complex in vitro. These results indicate that FGF23 is quite unique among other members of FGF family. First, FGF23 is a hormone regulating mineral homeostasis rather than working as a local factor involved in the modulation of cell growth and differentiation like other members of FGF family. Second, although many mutations of FGF receptor are reported, both activating and inactivating mutations of *FGF23* gene are known to cause human diseases as ligand for FGF receptor. Finally, FGF23 requires klotho for its signaling in addition to FGF receptor. It is likely that FGF family members have broader biological functions and mode of actions than previously recognized.