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NEW HORIZONS IN

New understanding and treatments for osteoporosis

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Abstract To summarize promising areas of investigation in osteoporosis and to stimulate further research in this area, as discussed in a recent international conference. Over the recent years, there has been an improvement in the knowledge of molecular pathways involved in bone formation and resorption with the development of new drugs to treat osteoporosis. Intact parathyroid hormone, teriparatide, and anti-sclerostin monoclonal antibody are anabolic drugs, whereas denosumab and odanacatib are anti-resorptive drugs with more reversible effects as compared to bisphosphonates. Anabolic and anti-resorptive agents have different effects on bone, and research in this area includes the efficacy of combination and sequential therapies with them. New insights in the molecular pathways of bone remodeling have clarified the mechanisms responsible for skeletal fragility in several forms of secondary osteoporosis, such as that occurring in type 2 diabetes, following drug exposure and systemic inflammatory diseases. Future research is needed to address the efficacy of anti-osteoporotic drugs in these more recently recognized conditions of skeletal fragility. Osteoporosis continues to be an important field of biomedical research.

Keywords Osteoporosis · Osteoblasts · Osteoclasts · PTH · RANKL · Cathepsin K · Sclerostin · Diabetes

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Introduction

Osteoporosis is a skeletal disorder characterized by a decrease in bone strength leading to an increased risk of fractures. About half of the women at age of 50 or older will suffer an osteoporotic fracture during their lifetime, causing disability, increased mortality, and financial burden [1]. Fragility fractures occur less commonly in men, but they are associated with a higher mortality than in women [2]. Factors that determine risk of osteoporosis and fractures include genetic, hormonal, and nutritional factors, and specific pharmacological therapies for immunological, and inflammatory disorders.

The 4th Skeletal Endocrinology meeting, held in Brescia on April 15, 2011, was established as a forum to facilitate interactions among World experts conducting research in osteoporosis, with a focus on hormonal actions in bone. This review summarizes new insights into the pathogenesis and treatment of osteoporosis and identifies areas of uncertainty in this field of study.

Novel insights into bone cell biology

FoxOs, oxidative stress and aging

The doubling of life expectancy in the last 200 years has made it important to know whether skeletal involution is an inexorable feature of longevity; and if so, whether it can be combated by targeting molecular pathways and mechanisms of aging. Reactive oxygen species (ROS) attenuate osteoblastogenesis and shorten the lifespan of osteoblasts and osteocytes. ROS are required for osteoclast generation, function, and survival [3]. Increased ROS generation leads to the activation of FoxOs, transcription factors that are an important defense mechanism against oxidative stress [4, 5]. Global deletion of FoxOs in young mice increases oxidative stress and recapitulates the adverse effects of aging on bone. Conversely, FoxO3 overexpression in mature osteoblasts decreases oxidative stress and increases bone mass. Furthermore, FoxO3 expression in cells of the osteoclast lineage decreases osteoclastogenesis, increases osteoclast apoptosis and bone mass. Both, aging and estrogen deficiency increase the generation of ROS, and there was evidence to suggest that adverse effects of estrogen loss on bone may be prevented by anti-oxidants [5]. Conversely, estrogens decrease oxidative stress and antagonize ROS-induced osteoblast apoptosis and the pro-survival effects of receptor activator of nuclear factor-κ B-ligand (RANKL) on osteoclasts [6]. It has been proposed that elucidation of these mechanisms provides a paradigm shift from the "estrogen-centric" account of the pathogenesis of involutional osteoporosis to one in which age-related mechanisms intrinsic to bone are central to the disease process [7].

Notch and the skeleton

Notch are transmembrane receptors that regulate cell fate decisions [8]. There are four Notch receptors in mammals (Notch 1 through 4). Upon binding by members of the Delta and Jagged family of transmembrane proteins, Notch is cleaved and the Notch intracellular domain (NICD) is released. NICD then translocates to the nucleus, where it associates with CBF-1, Suppressor of Hairless, Lag-2 (CSL), also termed RBPJKappa, and with Mastermind-Like (MAML) proteins [9]. This complex activates the transcription of target genes, such as Hairy Enhancer of Split (Hes) and Hes-related with YRPW motif (Hey). Notch plays a fundamental role in cell fate and function [10]. The effect of Notch is cell-context dependent, and in undifferentiated cells of the osteoblastic lineage Notch inhibits osteoblastic differentiation, causing severe osteopenia [11]. Conditional inactivation of Notch in osteoblasts leads to increased osteoclastogenesis and bone loss, suggesting that Notch suppresses osteoclast formation [12]. But, Notch plays a role in tumor invasion and bone metastasis, confirming that the cellular environment dictates Notch actions [13, 14]. Notch regulates Wnt signaling; and since Wnt inhibits adipogenesis, Notch may play an indirect role in the differentiation of adipocytes. In addition, Notch interacts with the nuclear factor of activated T cells (NFAT) signaling network [15]. The Notch ligand Jagged 1 is induced by parathyroid hormone (PTH), and Notch mediates effects of this hormone in the hematopoietic niche. Dysregulation of Notch signaling is the underlying cause of developmental and acquired diseases affecting the skeletal tissue, including Alagille syndrome, osteosarcoma, and Hajdu-Cheney Syndrome, a disease characterized by marked osteoporosis due to gain of Notch 2 function [8, 16, 17].

The calcium sensing receptor and bone homeostasis

Bone cells, particularly osteoblasts and osteoclasts, respond to calcium. Recent studies provide strong evidence that the calcium sensing receptor (CaSR) is expressed in osteoblasts, osteoclast precursors and in mature osteoclasts [18]. Both in vitro and in vivo studies indicate that the CaSR regulates bone cell metabolism. The CaSR is involved in the recruitment, differentiation and survival of osteoblasts and enhances bone formation and mineralization [19]. Moreover, the CaSR appears to have a permissive role in osteoclastogenesis, even though at high concentrations calcium inhibits osteoclast activity and causes apoptosis [20]. These data suggest that the CaSR might be involved in skeletal homeostasis by sensing changes of calcium in the skeletal microenvironment [21]. Indeed, bone resorption induces local increases in calcium within the immediate vicinity of osteoclasts and could provide pre-osteoblasts with a signal that modulates their subsequent physiological responses, such as migration and proliferation. Monocytes–macrophages have the capacity to fuse and differentiate into mature functional osteoclasts. Bone marrow stromal cells could modulate these processes by sensing high calcium in the skeletal microenvironment. On the other hand, high calcium-induced increases in cytosolic calcium concentration in mature osteoclasts could promote osteoclast apoptosis and inhibition of bone resorption. Emerging research indicates a role of CaSR in the hematopoietic stem cell niche, with implications for bone marrow transplant, and in skeletal metastases from breast and prostate cancer [22].

Drugs that target the CaSR directly either as agonists (calcimimetics) or antagonists (calcilytics) have been developed, but their therapeutic potential is hampered by their effects on non-skeletal tissue [23, 24]. Targeting the bone CaSR using bone-seeking CaSR agonists will offer a

tool to modulate bone cell metabolism. Such a drug might be useful in the treatment of osteoporosis.

Parathyroid hormone and the immune system

Intermittent administration of PTH, an approved treatment of osteoporosis, improves bone microarchitecture and strength [25]. Conversely, primary hyperparathyroidism, a common metabolic bone disease, is associated with bone loss. The underlying cellular and molecular mechanisms that can lead to bone loss or gain in response to the same hormone are unclear. T cells also express PTH receptors suggesting that they may be a target of PTH [26]. T cells provide proliferative and survival cues to stromal cells and sensitize them to PTH through CD40 Ligand, a surface molecule of activated T cells that induces CD40 signaling in stromal cells [27]. As a result, deletion of T cells or T cell-expressed CD40 Ligand blunts the catabolic activity of PTH in bone by decreasing bone marrow stromal cell number, RANKL/osteoprotegerin production and osteoclastogenic activity [28]. Accordingly, silencing of the PTH receptor in T cells also blocks the bone loss and the osteoclastic expansion induced by continuous PTH, thus demonstrating that PTH signaling in T cells is central to PTH-induced bone loss. T cells also play a role in the bone anabolic effect of intermittent PTH, which is reduced in T cell deficient mice. The mechanism involves activation of Wnt signaling by T cells in pre-osteoblasts. Thus, T cell mediated activation of Wnt signaling in osteoblastic cells plays a permissive role in the mechanism by which intermittent PTH increases bone mass, suggesting that T cell osteoblast cross-talk pathways may provide pharmacological targets for bone anabolic therapies.

PTH: novel insights and approaches

Intact PTH and its 34 amino acid fragment known as PTH(1–34) or teriparatide are effective treatments for osteoporosis [29, 30]. These osteoanabolic agents stimulate bone formation by affecting bone modeling and by stimulating bone remodeling. The effects on modeling lead to increased bone formation whereas the effects on bone remodeling lead to increased bone turnover. The concept of the anabolic window is derived from these properties when at least for a limited period of time the effects of PTH on bone are primarily anabolic [31]. Implicit in this concept is the idea that the osteoanabolic effects of PTH are not sustained. There is little evidence to support this concept because most clinical trials have been limited to 2 years. A recent trial in glucocorticoid-induced osteoporosis was conducted for a period of 3 years, providing insight into the consequences of longer term use of teriparatide [32, 33]. While bone mineral density (BMD) continued to accrue over the 3 year period, most notably in the lumbar spine, and fracture reduction in comparison to alendronate was sustained for 3 years, bone turnover markers declined toward baseline at the end of the study. These results are consistent with the hypothesis that effects of PTH on bone turnover are limited, but do not address the possibility of a long-term benefit on fracture reduction, which could be sustained beyond the period of PTH administration.

The notion that PTH when given as an intermittent low dose treatment, has a limited time period of action has led to approaches in which the drug is given for a short period of time followed either by no intervening therapy or by a bisphosphonate [34–37]. When patients are retreated with PTH there seems to be a robust response whether or not they are on bisphosphonate in the interim period of time. Whether this approach is more practical, feasible or better than the use of PTH for 2 continuous years as approved by regulatory agencies remains to be determined.

Various approaches to improve the effects of intact PTH or teriparatide have employed combinations with an anti-resorptive, such as a bisphosphonate [38]. The rationale for this approach is that anti-resorptive and anabolic therapies act by different mechanisms. By inhibiting bone resorption, the resorptive effects of PTH would be mitigated, thus magnifying its actions on bone formation. Despite the attractiveness of this concept, the results of simultaneous anti-resorptive and PTH therapy have not shown an advantage over monotherapy with either PTH or the anti-resorptive alone [36–40]. The most recent attempt in this regard was reported by Cosman et al. [41], in which daily teriparatide was used in combination with a single infusion of zoledronic acid. While there seemed to be a densitometric advantage of the combination teriparatide-zoledronic acid in the first 6 months, at 12 months combination therapy had no advantage when compared to the effects of either teriparatide (lumbar spine) or zoledronic acid (hip) alone on BMD.

A potential application of teriparatide or PTH(1–84), currently under investigation, is in the treatment of hypoparathyroidism [42]. Hypocalcemia is its main biochemical abnormality. Standard treatment regimens include the administration of calcium and vitamin D. Hypoparathyroidism is the only classic endocrine deficiency disease for which the deficient hormone (i.e., PTH) is not approved for replacement therapy. A number of studies, however, show that both teriparatide or PTH(1–84) provide good calcemic control, while reducing the intake of calcium and vitamin D in these individuals [43–45]. In addition, urinary calcium excretion falls. Because of its relatively short half-life, teriparatide is given multiple times daily, while a daily injection of PTH(1–84) has been sufficient to control hypocalcemia. PTH molecules in which specific sequences

have been altered to prolong its biological half-life are being developed. Such PTH molecules have the potential to provide a lasting control of serum calcium in hypoparathyroidism, but currently data are lacking to determine whether this approach is feasible and safe.

RANKL–OPG system and bone: new clinical developments

Several metabolic bone diseases are caused by excessive bone resorption resulting in bone loss. Osteoclasts resorb bone, and in recent years an essential cytokine system for osteoclast generation has been characterized [46]. This system consists of 3 components: a ligand, RANKL, a cellular receptor, RANK, and a soluble decoy receptor, osteoprotegerin (OPG). RANKL binding to RANK is required for the differentiation of osteoclast precursors to mature cells, the survival of osteoclasts and the activation of mature osteoclasts to initiate bone resorption [46]. OPG, produced by osteoblasts and marrow stromal cells binds to RANKL, blocking its biological activity by preventing its association with RANK. As a result, OPG inhibits RANKL-induced bone resorption [46, 47]. *Opg*-null mice develop osteoporosis and fragility fractures [48]. Abnormalities of the RANK–RANKL–OPG system with an unbalanced increase in RANKL activity have been implicated in the pathogenesis of various skeletal diseases characterized by increased bone resorption. These diseases include various forms of osteoporosis, malignant bone tumors, primary hyperparathyroidism, and bone diseases secondary to inflammation.

Post-menopausal osteoporosis

Estrogens stimulate the expression of OPG from osteoblasts and blunt the responsiveness of RANK receptors by repressing downstream signals in osteoclasts [49]. The decreased estrogen production that occurs in post-menopausal women is accompanied by an unbalanced RANKL–RANK activity responsible for the increase in bone resorption and bone loss. The first evidence that targeting the RANKL–RANK system may be effective for the treatment of post-menopausal osteoporosis came from experimental studies in which the administration of OPG IgG-Fc fusion protein (OPG-Fc) improved trabecular structure and bone strength in ovariectomized rats [50]. Human observations confirmed that blocking the RANKL–RANK system by recombinant OPG-Fc was effective in the treatment of post-menopausal osteoporosis [51]. However, the formation of neutralizing antibodies against OPG after administration of the fusion protein, and its potential cross-reactivity with tumor necrosis factor-related apoptosis-inducing ligand (TRAIL), led to a new strategy. The development is denosumab, a human monoclonal antibody (IgG2 immunoglobulin isotype) which binds with high affinity and specificity to RANKL [52]. This drug is administered as a 60 mg subcutaneous injection at 6 month intervals. A single administration of denosumab induces a rapid (as early as 12 h) and profound (as much as 84%) inhibition of bone resorption which remains suppressed for as long as 6 months [53]. The suppression of bone formation occurs 1 month following the decrease in bone resorption [53]. A feature distinguishing denosumab from bisphosphonates is the rapid reversibility of its anti-resorptive effect [54]. Another difference between denosumab and bisphosphonates is that it can be used in patients with impaired renal function. However, in patients with severe renal impairment (creatinine clearance <30 ml/min) or on chronic hemodialysis, there is a risk of developing hypocalcemia [54].

In women with post-menopausal osteoporosis, the pivotal fracture trial reported by Cummings et al., demonstrated significant vertebral, non-vertebral, and hip fracture reduction over 3 years with denosumab [55]. A post hoc analysis demonstrated that denosumab was effective in preventing vertebral and hip fractures even in patients at higher risk as defined as those with pre-existing multiple or moderate-severe vertebral fractures, those aged 75 years or older and those with femoral neck T-scores of −2.5 SD or less [56]. The prevention of vertebral fractures was observed as early as after 12 months of treatment [55]. Denosumab was associated with an increased incidence of cellulitis when it was reported as a serious adverse event, although the recent extension of the pivotal fracture trial did not reveal an increased risk of skin infections. The Food and Drug Administration in the United States cautions against the use of denosumab in immunocompromised patients [57].

Drug-induced osteoporosis

Androgen-deprivation therapy is first-line therapy for metastatic prostate cancer although it is frequently used in men with non-metastatic prostate cancer. Androgen deprivation therapy is achieved with GnRH analogs alone or in combination with anti-androgenic therapy. GnRH analogs reduce serum testosterone and estradiol levels and increase bone turnover and bone loss [58]. Two years of treatment with denosumab in patients under androgen-deprivation therapy led to an increase in BMD at the lumbar spine, distal radius, and total hip by 6.7%, 5.5%, and 4.8%, respectively, with a 69% decrease in the incidence of vertebral fractures [59]. The anti-fracture efficacy of denosumab occurred as early as after 12 months of treatment and was sustained for 3 years [59].

Denosumab was also tested in women receiving aromatase inhibitors as adjuvant therapy of estrogen-receptor positive breast cancer [60]. Aromatase inhibitors prevent the aromatization of androgens and their conversion to estrogens in peripheral tissues. The dramatic reduction in estrogen concentrations caused by the suppression of androgen aromatization causes bone loss. Letrozole and anastrazole, which are non-steroidal aromatase inhibitors increase bone turnover, decrease BMD and increase the risk of vertebral and non-vertebral fractures by about 30% [58]. Two years of treatment with 60 mg of denosumab every 6 months led to an increase by 6% in BMD at the lumbar spine versus a loss of 1.5% in the placebo group [60]. No fracture data are available on the efficacy of denosumab in the treatment of aromatase inhibitor-induced osteoporosis.

Bone disease in neoplasia

Osteoclasts and RANKL/RANK/OPG pathway are the target of malignant tumors capable of forming skeletal metastases or causing hypercalcemia [61]. RANKL-mediated mechanisms have been described for a variety of osteotropic malignancies, such as breast, lung, and prostate cancer. Moreover, an enhanced and uncontrolled osteoclastic bone resorption was described in patients with multiple myeloma in close relationship with the osteolytic lesions [62]. Neoplastic cells may stimulate bone resorption by releasing cytokines capable of enhancing RANKL production by stromal cells [63]. Moreover, some neoplastic cells, such as myeloma cells, inhibit OPG production leading to an unbalanced activation of RANK pathway [64]. Activated osteoclasts produce cytokines that stimulate the growth of tumor cells creating a vicious cycle of bone destruction [65]. These experimental findings offer the rationale for targeting RANK–RANKL–OPG in the prevention of skeletal-related events occurring in patients with bone lesions from several tumors. Denosumab at 120 mg every 4 weeks was shown to be more effective at delaying skeletal-related events, such as pathological fractures and spinal cord compression or the need for radiation or surgical treatment, than zoledronic acid in patients with bone metastasis from breast and prostate cancer [66, 67]. Moreover, denosumab was shown non-inferior to zoledronic acid in preventing or delaying skeletal-related events in patients with multiple myeloma or bone metastases from solid tumors [68]. Based on this evidence, denosumab at dosage of 120 mg every 4 weeks is approved for the treatment of patients with bone metastasis from solid tumors, offering a therapeutic alternative to intravenous bisphosphonates for patients with bone metastases.

Bone disease in rheumatoid arthritis

The skeletal complications of rheumatoid arthritis include focal bone erosions, juxta-articular osteopenia at sites of active inflammation and systemic osteopenia, resulting in significant joint deformity, disability and pain in addition to an increased risk of bone fractures [69]. There is convincing evidence that osteoclasts play a key role in the pathogenesis of bone loss occurring in rheumatoid arthritis. Moreover, there is evidence of cross-talk between T lymphocytes involved in the pathogenesis of arthritis and osteoclasts which drive bone resorption leading to bone loss in rheumatoid arthritis [70]. The mechanism involves the RANKL–RANK–OPG system since activated T lymphocytes express and produce RANKL which in turn is responsible for driving osteoclastogenesis and bone resorption [70]. Therefore, targeting the RANKL–RANK–OPG system may be effective in preventing bone damage in patients with rheumatoid arthritis. In an experimental model of arthritis, administration of recombinant OPG-Fc reduced bone loss and cartilage destruction [71]. In humans, denosumab at 60 and 180 mg every 6 months inhibited the progression of bone erosion in patients with active rheumatoid arthritis, who were concomitantly receiving treatment with methotrexate [72]. Denosumab did not reduce inflammation and disease activity.

Open issues

Several unresolved issues are the object of ongoing research: (1) A clinical trial is investigating the anti-fracture efficacy of denosumab in men with osteoporosis; (2) It is important to consider that because of the role of RANKL in T cell biology, denosumab could have adverse effects in the immune system [73]; (3) The safety of the long-term use of denosumab, possibly leading to prolonged suppression of bone turnover and to osteonecrosis of the jaw (ONJ) (2 cases crossed-over from the 3-year placebo arm in the 5 year denosumab extension trial for osteoporosis were reported to have ONJ), especially in predisposed patients, such as those with neoplasia. Although, the administration of recombinant OPG-Fc was shown to be effective in preventing bone metastasis in experimental animals with solid tumors [74], it is unknown whether denosumab may have similar efficacy in humans.

New therapeutic horizons

New knowledge of bone pathophysiology, bone remodeling and bone intracellular signaling pathways allowed identification of new treatment targets. Under development

are anti-catabolic drugs that target novel signals to inhibit bone resorption, such as cathepsin K inhibitors.

Cathepsin K is a protease, expressed in osteoclasts that contributes to the breakdown of bone matrix. Mutations in *CATHEPSIN K* gene cause pycnodysostosis, a rare bone disease characterized by osteosclerosis and suppressed bone resorption [75]. Three inhibitors of cathepsin K are balicatib, whose development was stopped because of adverse effects due to lack of specificity inhibition of other cathepsins, odanacatib, and ONO5334 [76, 77].

A phase II randomized double-blind placebo-controlled trial was reported in post-menopausal women by Bone et al. [78]. Odanacatib administration induced progressive dose-related increases in BMD. With the highest dose of 50 mg a week, lumbar spine and total hip BMD increased by 5.5 and 3.2%, respectively, when compared to placebo. Biochemical markers of bone turnover exhibited dose-related changes. The results of the extension of the phase II study (36 months) reported continued increases in BMD (lumbar spine 7.5%, total hip 5.5%, femoral neck 5.5%, and trochanter 7.4%) by odanacatib. Markers of bone resorption tended to be lower compared with placebo. After the discontinuation of odanacatib, the effects on BMD were rapidly lost. The safety and tolerability of odanacatib was similar to that of placebo. Odanacatib seems to act differently from other anti-resorptive agents, in that it does not reduce osteoclast number. Moreover, odanacatib appears to reduce bone formation to a lesser extent than bisphosphonates and denosumab. A phase III trial is currently in progress.

The identification of new pathways involved in bone formation is leading to the development of new anabolic agents. Sclerostin, the protein encoded by the *SOST* gene, mainly expressed in osteocytes, is a key negative regulator of bone formation acting through the inhibition of Wnt signaling [25]. The expression of *SOST* is downregulated by PTH [25]. In humans, mutations of *SOST* lead to sclerosteosis and van Buchem disease, which are characterized by increased bone mass. Sclerostin deficiency reproduces the findings of the human diseases in mice while sclerostin excess leads to bone loss and reduced bone strength [79]. In a rat model of post-menopausal osteoporosis due to ovariectomy, treatment with a sclerostin antibodies increased bone mass at all skeletal sites and prevented the bone loss associated with estrogen deficiency [80]. Administration of two once-monthly injections of a humanized sclerostin-neutralizing monoclonal antibody to intact female monkeys increased bone mineral content and BMD at several skeletal sites. In addition, significant increases in trabecular thickness and bone strength were found in the lumbar vertebrae with higher doses [81]. In a phase I, ascending, single dose study performed in healthy men and post-menopausal women, sclerostin monoclonal antibodies were generally well tolerated [82]. Dose-related increases in the bone formation markers procollagen type I N propeptide (P1NP), alkaline phosphatase and osteocalcin were observed, along with a dose-related decrease in the bone resorption marker serum C-telopeptides of type-1 collagen (CTX). Increases in BMD at the lumbar spine and at the total hip were also observed. A phase II trial has been started to compare the efficacy of sclerostin neutralization with alendronate and teriparatide.

Increased Wnt signaling has been associated with tumors, and Wnt inhibitory factor 1 was silent in 75% of human osteosarcomas, leading to enhanced Wnt signaling [83, 84]. Although patients with sclerosteosis and van Buchem disease do not appear to have an increased risk of malignancy, careful studies are needed for monitoring skeletal and extra-skeletal safety of a long-term blockade of Wnt antagonists.

Selected clinical topics

Long-term safety of bisphosphonates

Bisphosphonates are the most commonly prescribed agents for the treatment of osteoporosis. Their efficacy in reducing fracture risk with a favorable safety profile is well-established. However, their long-term use has been associated with reports of undesirable events not previously recognized. These include ONJ and atypical femoral fractures. Early reports associating bisphosphonate use with esophageal cancer or atrial fibrillation have not been substantiated.

A systematic review published by Khan et al. [85] showed that high-dose intravenous bisphosphonates in oncologic patients is associated with an increased risk of ONJ that appears to be dependent on dose and duration of therapy. This relationship has not been confirmed with lower-dose bisphosphonate therapy given to osteoporotic patients who likely show a frequency of ONJ that is modestly different from the general population. The lesions in the latter patients appear to be less severe and more likely to resolve than those found in patients receiving high doses of intravenous bisphosphonates. Based on PROBE study, it appears that the prevalence of ONJ may increase with increased duration of therapy (4 or more years) [86]. Recently, the Food and Drug Administration in the United States modified the labels of bisphosphonates to clarify the recommended duration of therapy and how often physicians should re-evaluate patients taking these drugs. Although the potential risk of ONJ is remote, Guidelines of the American Association of Oral and Maxillofacial Surgeons suggest that discontinuation of oral bisphosphonates 3 months before and 3 months after invasive oral surgery is reasonable [87].

The question of whether atypical femoral fractures are causally related to bisphosphonate therapy is widely debated, but as yet unresolved. The true incidence of atypical femur fractures in patients with and without bisphosphonate treatment is unknown, although they appear to occur more often in patients on long-term therapy (greater than 5 years), independently of the dose administered [88]. Regardless, these fractures are rare, particularly in proportion to the number of fractures prevented by therapy.

Preliminary reports of esophageal cancer in patients on bisphosphonate treatment triggered investigation into this matter. Although some studies reported no difference in the risk of esophageal cancer between patients and control subjects, others have shown an increased risk [89]. However, the evidence that bisphosphonates are etiologically associated with esophageal cancer is not compelling.

The incidence of atrial fibrillation, reported as a serious adverse event, was increased in the pivotal trial testing the effect of zoledronic acid in post-menopausal women with osteoporosis. Subsequent studies have not confirmed this finding with zoledronic acid or other bisphosphonates. Moreover, there is no evidence of increased mortality or increased risk of stroke [90]. The lack of an association between bisphosphonates and atrial fibrillation extends to their use in oncology where the bisphosphonate dose is typically up to 10 times the dose used for osteoporosis. In a recent meta-analysis of all placebo controlled clinical trials of alendronate, the occurrence of atrial fibrillation was uncommon and no clear association between bisphosphonate exposure and the rate of serious and non-serious atrial fibrillation was observed [91].

In conclusion, serious adverse effects associated with bisphosphonates in post-marketing reports appear to be rare in relationship to the benefit of reducing fracture risk. A careful evaluation of the balance between the expected benefit and potential risk should guide the decision to initiate a treatment with these drugs and the duration of therapy.

Diabetes mellitus and bone

Diabetes mellitus is a chronic disease causing several complications, including retinopathy, nephropathy, cardiomyopathy, neuropathy, and peripheral artery disease. Recent evidence from epidemiological and clinical studies suggests that diabetes mellitus is also a risk factor for osteoporosis and fragility fractures. This association is of clinical interest, considering the high mortality risk demonstrated in both osteoporosis and diabetes.

Several factors may be involved in the pathogenesis of osteoporosis and fractures in diabetes mellitus. Hyperglycemia may have direct and indirect deleterious effects on osteoblast function and bone formation. High glucose concentrations suppress osteoblast growth and differentiation and stimulate adipogenesis [92]. Moreover, hyperglycemia may cause an increase in the expression of osteoclastogenic mediators, such as tumor necrosis factor-alfa (TNF-alfa) and RANKL, with a consequent stimulation of bone resorption [93]. Bone strength is impaired with an increase in advanced glycation end products and non-enzymatic cross-links within collagen fibers caused by hyperglycemia with a consequent deterioration in the structural and mechanical properties of bone [94]. Advanced glycation end products may also induce osteoblast apoptosis [95]. Insulin and insulin-like growth factor-1 exert anabolic effects on bone, and the absence or decrease of these hormones in type 1 diabetes may contribute to bone loss by an impairment of osteoblastic function [96]. Chronic vascular complications may be predisposing factors to osteoporosis in diabetes [97]. In addition to the impaired bone strength, patients with diabetes are predisposed to fractures due to the propensity to fall, mediated through impaired vision, impaired proprioception and recurrent hypoglycemic crises.

Most studies have reported decreased BMD in adults as well as in children with type 1 diabetes, with osteopenia and osteoporosis occurring in up to 60 and 20% of the patients, respectively [97]. Consistent with the changes in BMD, patients with type 1 diabetes mellitus are at high risk of fragility fractures, with odds ratio for hip and vertebral fractures being 1.7 and 2.5, respectively [98]. A normal to increased BMD has been reported in patients with type 2 diabetes, and it is not entirely explained by overweight [99]. However, an increased risk of fragility fractures occurs in patients with type 2 diabetes, confirming that fractures in secondary osteoporosis cannot be predicted by BMD [99–106]. The risk of fractures in type 2 diabetes is lower than in type 1 diabetes but affects both vertebral and non-vertebral sites [98, 107]. It is noteworthy that fracture risk was not found to be significantly correlated with the duration of diabetes, suggesting that fractures may be an early complication of the disease [98]. This could be of clinical significance in patients with type 2 diabetes, who may have been asymptomatic for prolonged periods of time before the diagnosis was made.

Presently, there are no specific guidelines for the diagnosis and treatment of osteoporosis in patients with diabetes mellitus. Calcium and vitamin D could be recommended and anti-resorptive agents can be used if indicated [108]. Experimental data suggest that metformin, insulin, glimepiride and incretins, such as glucose dependent insulinotropic polypeptide and glucagon-like peptide-1, may have beneficial effects on bone remodeling unrelated to their effects on glucose control [109–112]. In contrast, there is evidence demonstrating that thiazolidinediones increase osteoporosis and fracture risk in patients with type 2 diabetes. Thiazolidinediones are insulin-sensitizing drugs acting

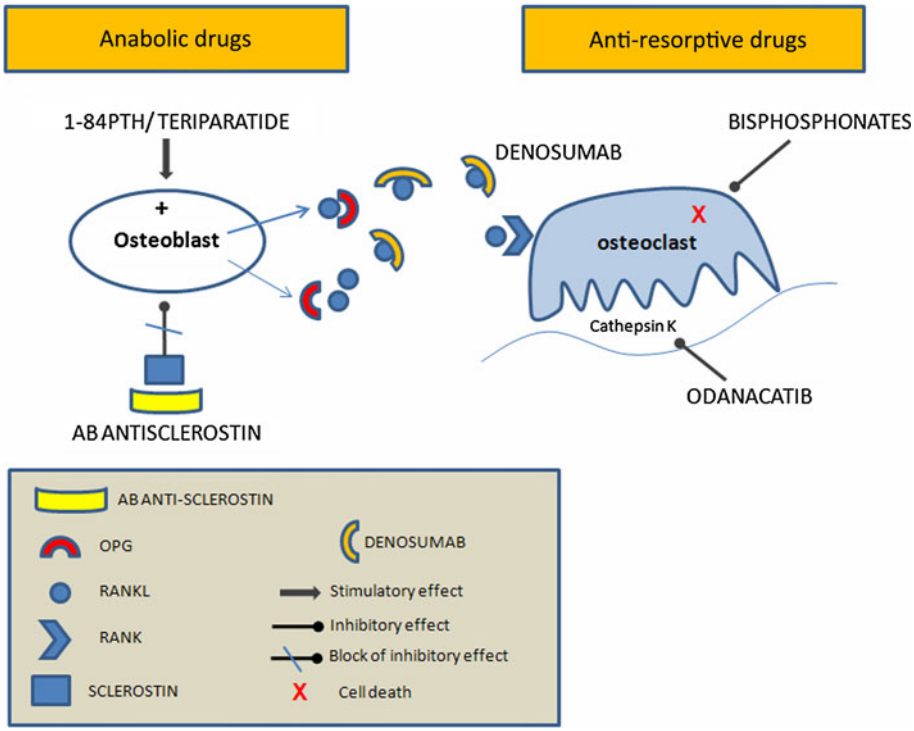

Fig. 1 Targets of anti-osteoporotic drugs; *OPG* osteoprotegerin, *RANK* nuclear factor-k B, *RANKL* nuclear factor-k B-ligand

as selective agonists of peroxisome proliferator-activated receptor (PPAR)γ. This receptor is expressed in marrow stromal cells, and PPARγ induction in these cells promotes adipogenesis at the expense of osteoblastogenesis, with the potential to inhibit bone formation and induce bone loss [25]. Thiazolidinediones may also influence skeletal health by modulating adipokine production by adipose tissue and influencing the growth hormone/insulin-like growth factor-1 axis and gonadal function with secondary effects on bone remodeling [113]. Recently, a series of clinical studies have confirmed that thiazolidinediones are associated with adverse skeletal effects [58]. A decrease in BMD with an increase in vertebral and non-vertebral fractures were described in patients treated with rosiglitazone, a drug no longer in use for the treatment of type 2 diabetes due to cardiovascular complications [114, 115]. Pioglitazone, another thiazolinedione, also was shown to be associated with an increased risk of fragility fractures [116, 117].

There are unresolved questions in this area: (1) The prediction of fractures, as well as the monitoring of treatment, may be a challenge in patients with type 2 diabetes, since BMD may not be useful in this specific clinical setting. Other surrogate measures of bone strength have been proposed, but their reliability in clinical practice needs to be confirmed [118]; (2) Since osteoporosis in diabetic patients is predominantly secondary to an impairment of bone formation, anabolic therapies may be preferable but their effectiveness needs to be assessed, and it is not known whether they are more effective than anti-resorptive agents in these patients [101]; and (3) It is unknown whether newly developed anti-diabetic agents, in particular the incretins, decrease fracture risk regardless of glycemic control in patients with type 2 diabetes.

Conclusions

Over the recent years, there have been new insights into bone cell biology with new knowledge of anabolic and resorptive pathways involved in bone remodeling (Fig. 1). Anabolic pathways responsible for osteoblastogenesis and bone formation have been extensively characterized. Anabolic drugs, such as intact PTH and teriparatide, have been introduced in clinical practice and others, such as anti-sclerostin monoclonal antibodies, are under investigation for their efficacy in the treatment of osteoporosis. Molecular mechanisms regulating bone resorption have been clarified permitting the development of novel anti-resorptive drugs, such as denosumab and odanacatib, which appear to have shorter biological half-lives than bisphosphonates.

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