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Molecular understanding of pharmacological treatment of osteoporosis

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- Osteoporosis is a serious health concern, particularly in aged societies. The burden of osteoporosis with its associated morbidity and mortality due to fracture has become a critical socioeconomic problem.
- Skeletal integrity is maintained through a balance of bone resorption and bone formation. The bone turnover process, called bone remodelling.
- Recently, a number of anti-osteoporosis drugs with excellent anti-osteoporosis and fracture effects have been developed. They are mainly classified into two groups according to their effects on bone remodelling: anti-resorptive agents and anabolic agents.

Keywords: abaloparatide; bisphosphonate; denosumab; oestrogen; osteoporosis; romosozumab; SERM; teriparatide

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Introduction

Osteoporosis is a serious health concern in the global community. In aged societies such as Japan and Italy, an increasing number of people are suffering from osteoporosis and osteoporotic fractures such as vertebral fractures and hip fractures. Approximately 75 million people are estimated to have osteoporosis in the United States (US), Europe and Japan,[1,2] and the burden of osteoporosis with its associated morbidity and mortality issues due to fractures has become a critical socioeconomic problem.

According to the US National Institutes of Health (NIH) consensus statement, osteoporosis is defined as a skeletal disorder characterized by compromised bone strength predisposing to an increased risk of fracture.[3] Bone strength is determined by two important features: bone density and bone quality. Bone mineral density is defined as the bone mineral content per projectional bone area (g/cm^2), while bone quality is related to various characteristics of bone, such as microarchitecture, bone turnover, microdamage accumulation, uniformity of mineralization, and collagen crosslink formation.

Skeletal integrity is maintained through a balance of bone resorption and bone formation. The bone turnover process, called bone remodelling, continues throughout life. Bone remodelling is a sequential process and starts with the 'activation' phase, defined as the conversion of bone surface from quiescence to active, which is followed by the differentiation of osteoclast precursors into mature osteoclasts in the 'resorption' phase. In the 'reversal' phase, osteoclasts complete the resorption process and produce signals that directly or indirectly initiate bone formation, and in the final 'formation' phase, mesenchymal cells differentiate into functional osteoblasts to make the bone matrix.[4,5] The length of the resorption phase is very short (2–4 weeks) compared with that of the formation phase (4–6 months), and the life span of osteoclasts is much shorter than that of osteoblasts. Therefore, increased bone remodelling necessarily leads to increased bone resorption and negative bone mass balance. Recker et al reported that the activation frequency reflecting the bone remodelling rate increased to double at menopause, triple by 13 years later, and remained at high levels in osteoporotic patients, which contributes to increases in age-related skeletal fragility in women,[6] confirming that high activation frequency is associated with the increased bone loss in oestrogen-deficient postmenopausal women.

Recently, a number of anti-osteoporosis drugs with excellent anti-fracture effects have been developed. They are mainly classified into two groups according to their effects on bone remodelling: anti-resorptive agents and anabolic agents.[7] Anti-resorptive agents suppress bone resorption, and therefore reduce bone remodelling, while anabolic agents enhance bone remodelling by increasing bone formation more than bone resorption. This review article will focus on the molecular understanding of the mechanisms of action of anti-osteoporotic drugs, although novel agents, such as abaloparatide, will not be described in detail because of the page limit. In addition, the use of combination therapy, such as treatment with both

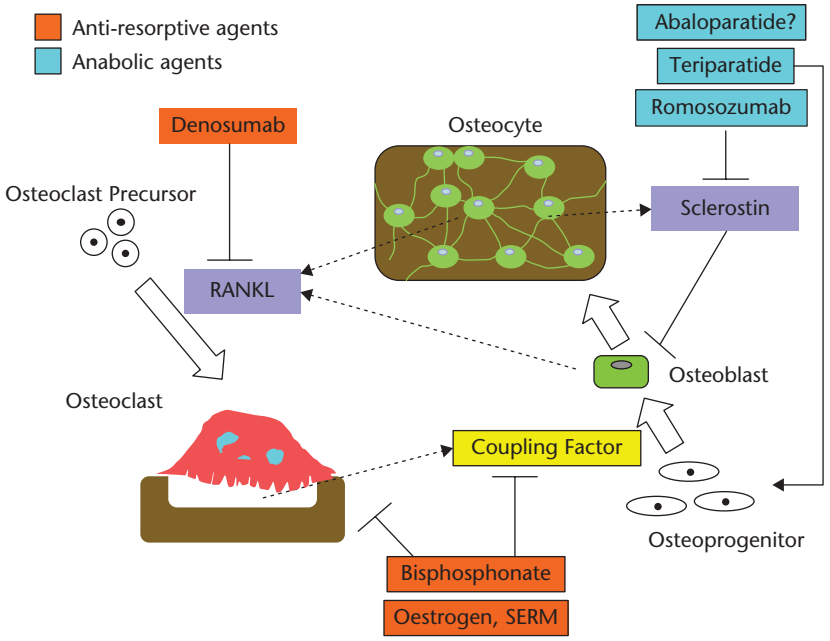

Fig. 1 Regulation of bone metabolism and mechanisms of action of anti-osteoporotic drugs.

teriparatide plus denosumab is not summarized herein. Fig. 1 summarizes the regulation of bone metabolism and mechanisms of action of anti-osteoporotic drugs, and Table 1 summarizes the anti-fracture evidence of each drug.

Bisphosphonate

Bisphosphonates, stable analogues of pyrophosphate, are the most commonly used anti-osteoporosis medicine with strong anti-resorptive activity, and the first-generation bisphosphonates such as etidronate, clodronate, and tiludronate are different from later-generation bisphosphonates such as alendronate, risedronate, ibandronate, pamidronate, and zoledronate in terms of the lack of a nitrogen-containing side chain.[8] Accumulating clinical evidence has demonstrated the anti-fracture effects of various types of bisphosphonates. Although bisphosphonates are poorly absorbed from the intestine when orally administered, they immediately bind to the mineralized surface of the bone matrix once they are absorbed into the bloodstream. There are some differences among bisphosphonates in their mineral-binding affinities that can affect their pharmacological and biological properties. Following bone resorption by osteoclasts, bisphosphonates are released from bone surfaces via the acidic environment generated by osteoclasts, and ingested by osteoclasts through endocytosis. Coxon et al beautifully visualized the process of internalization of bisphosphonates by osteoclasts using fluorescently labelled bisphosphonates.[9] Once taken up by the bone, the half-life of bisphosphonates largely depends on the rate of bone turnover, and the half-life of alendronate in bone has been estimated to be greater than 10 years for humans.[10]

The molecular mechanisms of action of bisphosphonates are now well understood. The first-generation bisphosphonates, such as etidronate and clodronate, are incorporated into non-hydrolysable analogues of adenosine triphosphate and induce osteoclast apoptosis. In contrast, second and third-generation nitrogen-containing bisphosphonates (aminobisphosphonates) suppress farnesyl pyrophosphate synthase, a key enzyme of the mevalonate pathway, inhibit the function of small G proteins such as Rho and Rac, and subsequently affect downstream intracellular signalling pathways.[11] Aminobisphosphonates bind to the dimethylallyl/geranyl pyrophosphate ligand pocket and induce a conformational change of farnesyl pyrophosphate synthase. We reported that risedronate, a third-generation bisphosphonate, efficiently suppresses the activity of extracellular signal-regulated kinase (ERK) and protein kinase B (also known as AKT) pathways in osteoclasts. Suppression of ERK upregulates the expression of the proapoptotic Bcl-2 family molecule Bim and induces apoptosis of osteoclasts, while suppression of AKT affects the cytoskeletal organization of osteoclasts and reduces the bone-resorbing activity.[12]

Denosumab

Receptor activator of nuclear factor kappa B ligand (RANKL) plays critical roles in osteoclast differentiation.

Table 1. Summary of anti-fracture evidence of osteoporosis drugs

Mode of action	Group	Agent	Effect on fracture reduction and level of evidence		
			Vertebral fracture	Nonvertebral fracture	Hip fracture
Antiresorptive	Bisphosphonate	Alendronate	↓; strong evidence	↓; strong evidence	↓; strong evidence
		Risedronate	↓; strong evidence	↓; strong evidence	↓; strong evidence
		Ibandronate	↓; strong evidence	↓; strong evidence	Not studied
		Zoledronic acid	↓; strong evidence	↓; strong evidence	↓; strong evidence
Antiresorptive	RANKL antibody	Denosumab	↓; strong evidence	↓; strong evidence	↓; strong evidence
Antiresorptive	Oestrogen	Conjugated estrogen	↓; strong evidence	↓; strong evidence	↓; strong evidence
Antiresorptive	SERM	Raloxifene	↓; strong evidence	↓; weak evidence	→; strong evidence
		Basedoxifene	↓; strong evidence	↓; weak evidence	→; strong evidence
Antiresorptive	Calcitonin	Salmon Calcitonin	↓; weak evidence	→; strong evidence	Not studied
Anabolic	PTH	Teriparatide	↓; strong evidence	↓; strong evidence	→; weak evidence
Anabolic		Abaloparatide*1	↓; strong evidence	↓; strong evidence	Not reported
Anabolic Antiresorptive	Sclerostin antibody	Romosozumab*2	↓; strong evidence	↓; strong evidence	↓; strong evidence

↓: decreased; →: no effect.
*1: Approved in the US.
*2: Approved in the US and Japan.

RANKL belongs to the tumour necrosis factor superfamily, and binds to its receptor RANK, expressed in osteoclast precursors. Osteoprotegerin (OPG), a member of the tumour necrosis factor receptor superfamily, binds to RANKL and competitively suppresses the binding of RANKL to RANK. The physiologic importance of the RANKL–RANK–OPG axis was confirmed by a series of mouse genetic studies. The targeted disruption of RANKL or RANK, as well as the overexpression of OPG, induced osteopetrosis in mice because of the defect in osteoclast differentiation, while deletion of the OPG gene or the overexpression of RANKL leads to a marked reduction in bone mass, mimicking osteoporosis.[13] Additionally, several human genetic diseases were identified as being associated with the RANKL–RANK–OPG axis. Osteopetrosis is a genetic disorder characterized by increased bone mass due to a decrease in bone resorption. Patients with RANKL or RANK gene mutations develop a distinct subgroup of recessive osteopetrosis disease with very few osteoclasts in the skeletal tissues.[14] In contrast, familial expansile osteolysis, early-onset Paget's disease of bone and expansile skeletal hyperphosphatasia are associated with gene mutations that cause enhancement of RANKL–RANK pathways, and thus increased bone resorption.[14]

Denosumab is a fully human IgG2a monoclonal antibody that specifically binds to human RANKL and inhibits the interaction between RANKL and RANK, therefore suppressing bone resorption. In the pivotal Fracture Reduction Evaluation of Denosumab in Osteoporosis Every 6 Months (FREEDOM) study, denosumab significantly reduced osteoporotic fractures in postmenopausal women with osteoporosis.[15] Denosumab significantly and continuously increased bone mineral density (BMD) and reduced the risks of vertebral, nonvertebral and hip fractures.[16] There was no increase in the risk of cancer, infection, cardiovascular disease, delayed fracture healing, hypocalcaemia, or osteonecrosis of the jaw. In addition to its anti-osteoporotic function, denosumab prevents skeletal-related events in patients with bone metastasis from solid tumours,[17] and suppresses bone erosion in rheumatoid arthritis patients.[18]

Using a murine anti-RANKL antibody, we investigated the effect of RANKL suppression in a murine model of osteoporosis.[19] A substantial and rapid decrease in bone resorption was observed immediately after anti-RANKL antibody administration into ovariectomized mice, and the bone resorption parameters were significantly reduced 1–2 days after treatment. In contrast, reductions in bone formation parameters were relatively slow, and a significant amount of bone formation was still observed six days after anti-RANKL antibody treatment. Using transmission electron microscopy, osteoclasts were observed to be highly vacuolated and had well-developed ruffled borders before treatment, while those subjected to two days of anti-RANKL antibody treatment appeared to be flattened and had relatively unruffled borders. In contrast, mature osteoblasts did not show any morphologic differences between days 0 and 2.[19] These results suggest that the discrepancy between the suppression of bone formation and resorption may contribute to the continuous increase in BMD in patients treated with denosumab.

Oestrogen and selective oestrogen receptor modulators

The importance of oestrogen in maintaining skeletal integrity is evidenced by the fact that osteoporosis is more common in women than in men and that fragility fractures markedly increase after menopause. Albright et al reported in the 1940s that oestrogen administration restored the negative calcium balance in postmenopausal women.[20] A marked reduction of oestrogen levels is

observed during the menopausal transition, which causes a rapid increase in bone turnover and reduction of bone mass, leading to osteoporosis. The rapid bone loss at menopause continues for about 10 years, and is followed by slow and continuous bone loss.[6]

The molecular mechanism of how oestrogen signalling maintains skeletal integrity was extensively investigated using a cell-type specific oestrogen receptor (ER) α knockout (KO), although the results are somewhat controversial. Almeida et al reported that ERα deficiency in osteoblast progenitors suppressed proliferation and differentiation of periosteal cells, leading to reduced cortical bone mass, while ERα deficiency in mature osteoblasts or osteocytes was not associated with cancellous or cortical bone mass changes.[21] In contrast, Melville et al reported that female mice lacking ERα in mature osteoblasts and osteocytes had decreased cortical and cancellous bone mass, whereas male ERαKO mice had equal or greater bone mass than the control mice.[22] Martin-Millan et al demonstrated that an ERαKO in osteoclast progenitors exhibited a two-fold increase in osteoclast progenitors and a number of osteoclasts in cancellous bone, which was associated with a decrease in cancellous bone mass.[23] Although these results suggest the direct effect of oestrogen on bone-forming cells, clinical evidence has shown that the action of oestrogen is anti-resorptive. The effect of ERα deficiency in osteoclasts was reported by Nakamura et al. They found that ERαKO female mice, but not male mice, exhibited trabecular bone loss, which was caused by reduced apoptosis of osteoclasts due to reduced levels of Fas ligand expression.[24] Miyauchi et al reported that oestrogen deficiency stabilizes hypoxia-inducible factor (HIF) 1α in osteoclasts, activates bone resorption and promotes bone loss.[25] These results suggest that oestrogen signalling may play cell-type- and cell-stage-specific roles in the skeletal milieu.

A number of clinical studies also support the effectiveness of hormone replacement therapy (oestrogen and progestin) in the treatment of osteoporosis. The Research in Women's Health Initiative (WHI) confirmed the usefulness of oestrogen in reducing osteoporosis fractures. Oestrogen plus progestin reduced the observed hip and clinical vertebral fracture rates by one third compared with a placebo, both nominally significant. The reductions in other osteoporotic fractures (23%) and total fractures (24%) were statistically significant. However, the WHI study was terminated early because of an increased risk of breast cancer, heart disease, stroke, and venous thromboembolism in the women taking oestrogen and progestin.[26]

Several selective oestrogen receptor modulators (SERMs), such as raloxifene and bazedoxifene, were developed to provide the benefits of oestrogen therapy without its unwanted side effects.[27] SERMs bind to the oestrogen receptor (ER) and exhibit oestrogenic or anti-oestrogenic activity depending on the cell and tissue types by interacting with co-activators or co-repressors of ER. Raloxifene, the first SERM that was developed as an anti-osteoporotic drug, increased the BMD by suppressing bone resorption and decreased vertebral fractures in postmenopausal women. However, it was proven that the anti-fracture effect of raloxifene and bazedoxifene was limited, and that they did not significantly prevent nonvertebral fractures or hip fractures.[27]

Parathyroid hormone

Teriparatide is a recombinant formulation of endogenous parathyroid hormone (PTH), containing a 34-amino-acid sequence at the N-terminus. Teriparatide is the first anabolic anti-osteoporosis drug with powerful BMD-increasing activity and anti-fracture effects.[28] Intermittent administration of teriparatide markedly increases trabecular BMD and improves bone quality. In contrast, the continuously high PTH levels observed in disease conditions such as primary hyperparathyroidism are associated with bone catabolism, and increased bone resorption and decreased BMD are mainly observed in the cortical area. The catabolic effect of teriparatide is considered to be mediated by teriparatide-induced RANKL expression in osteoblasts and/or bone marrow stromal cells, which leads to increased osteoclastogenesis. The anabolic effect of teriparatide is at least partially mediated by the direct effect of teriparatide on osteoblast lineage cells because teriparatide increased bone turnover markers and BMD even in osteoclast-deficient RANK KO mice, although the BMD increase in KO mice was less than that in control mice. Additionally, the clinical observation that the teriparatide-induced BMD increase is dampened by co-treatment with bisphosphonates clearly indicates that the anabolic action of PTH is also mediated by coupling-induced bone formation, which is driven by precedent bone resorption.[29] Consistent with these speculations, Dempster et al recently reported that both remodelling-based and modelling-based bone formation were increased by teriparatide in human transiliac bone biopsies.[30]

In several countries including Japan, a once-weekly regimen of teriparatide is also approved for the treatment of osteoporosis. Interestingly, once-weekly teriparatide exhibits a different pattern of bone turnover marker changes from daily teriparatide, with an increase in bone-formation markers accompanied by a reduction in bone-resorption makers.[31] Additionally, the increase in bone-formation markers gradually decreases after four weeks and falls below baseline levels thereafter. Although the dynamics of bone turnover markers differ between daily and weekly teriparatide, increases in BMD and reductions in incident vertebral fracture are comparable, which cannot be explained by the 'anabolic window' theory. Therefore, we

hypothesized that repeated weekly teriparatide administration stimulates bone remodelling, replacing old bone with more new bone, which leads to a reduction in the active remodelling surface, and developed a simulation model.[32] The simulation model matched well with the actual change in bone turnover markers and BMD of patients treated with once-weekly teriparatide. Therefore, we concluded that remodelling-based bone formation persisted during the entire treatment period with once-weekly teriparatide administration.

The mechanism responsible for how teriparatide exerts its anabolic action on bone remains elusive. Mouse genetic studies have revealed the important roles of various molecules such as c-Fos, Runx2, and insulin-like growth factor in the bone anabolic action of PTH. We previously reported that the anti-apoptotic gene *bcl-2* plays a critical role in the teriparatide-induced BMD increase using *bcl-2* KO mice.[33] It was also demonstrated that the *SOST* gene and its protein product sclerostin, a potent negative regulator of bone formation that is mainly expressed in osteocytes, is implicated in PTH-induced bone gain, which will be described in detail below.

Recently, abaloparatide, a PTH-related peptide analogue that specifically activates PTH receptor type I pathway was developed as another anabolic drug,[34] and in phase 3 Abaloparatide Comparator Trial In Vertebral Endpoints (ACTIVE) trial, treatment with abaloparatide for 18 months significantly increased BMD and decreased the risk of vertebral, nonvertebral, clinical, and major osteoporotic fractures compared with a placebo.[35] Abaloparatide is approved in the US for the treatment of osteoporosis, and is specifically indicated for the treatment of postmenopausal women with osteoporosis at high risk for fracture.

Romosozumab

Recent studies have demonstrated the essential role of Wingless-type mouse mammary virus integration site (Wnt) pathways in osteoblastic bone formation. Wnt signalling is mainly associated with two types of pathways: canonical and noncanonical.[36] In the canonical pathways, Wnt ligands bind to co-receptors consisting of low-density lipoprotein receptor protein 5 and 6 (LRP5/6) and Frizzled, which are expressed in osteoblasts. Ligand binding to these receptors stimulates intracellular signalling cascades, which lead to the stabilization and nuclear translocation of β-catenin, and induction of downstream molecules involved in osteoblast differentiation, maturation, and survival. Sclerostin encoded by the *SOST* gene binds to LRP5/6, and inhibits downstream signalling of canonical Wnt pathways, thereby negatively regulating osteoblast function.[37] Additionally, sclerostin inhibits osteoclast differentiation by inducing OPG expression in osteoblasts and osteocytes. The critical function of sclerostin in bone homeostasis was further supported by the finding that genetic disorders with high bone mass phenotypes such as sclerosteosis and van Buchem disease are caused by defective synthesis of sclerostin. Furthermore, targeted disruption of the *Sost* gene in mice showed a high bone mass phenotype, mimicking these human diseases. These results clearly demonstrated that sclerostin is a negative regulator of bone formation by suppressing Wnt pathways.

Immunohistochemical studies have revealed that sclerostin is predominantly expressed in osteocytes although its expression was also detected in various tissues and cells. It was demonstrated that the expression of sclerostin in osteocytes is regulated by PTH through PTH/PTHrP receptor type 1. The anabolic effect of PTH was reduced in *Sost*-deficient mice.[38] Mechanical stress also negatively regulates *SOST* expression in osteocytes. Additionally, *Sost* KO mice did not exhibit bone loss by mechanical unloading, and conversely, loading-induced bone formation was greatly reduced in transgenic mice that constitutively expressed high levels of *Sost*.[39,40]

Based on the finding that the Wnt–sclerostin axis critically regulates bone formation, an antibody against sclerostin was developed as a novel anabolic agent for osteoporosis treatment. Romosozumab is a monoclonal antibody that binds and inhibits sclerostin, with dual effects of increasing bone formation and decreasing bone resorption. Clinical trials demonstrated that romosozumab markedly increased bone formation and therefore BMD, and suppressed vertebral fractures compared with a placebo in postmenopausal women with osteoporosis.[41] Additionally, romosozumab treatment for 12 months followed by alendronate resulted in a significantly lower risk of fracture than alendronate alone in postmenopausal women with osteoporosis who were at high risk for fracture.[42] One concern with this treatment was that there was a higher prevalence of serious cardiovascular adverse events observed in the romosozumab group compared with the alendronate group (2.5% vs 1.9%; odds ratio, 1.31).[42] Thus, romosozumab is a promising new anabolic agent with great anti-osteoporotic activity although adverse effects should be carefully monitored. Recently, romosozumab was admitted for the treatment of osteoporosis in patients at high risk of fracture in Japan, and postmenopausal women at high risk of fracture in the US.

Conclusion

This review article focused on the molecular understanding of the mechanisms of action of currently available as well as emerging anti-osteoporotic drugs. Understanding the mechanism of action of anti-osteoporotic drugs will be potentially useful for the efficacious and safe treatment

of osteoporosis patients, and future development of novel therapeutics.

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ICMJE CONFLICT OF INTEREST STATEMENT

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