Potential new drug targets for osteoporosis

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INTRODUCTION

Osteoporosis is a worldwide health problem that affects as many as 75 million people in the US, Japan and Europe. Fracture is the most common complication of osteoporosis—it is estimated that 30–50% of women and 15–30% of men with this disease have a fracture during their lifetime. Osteoporosis accounts for more hospital days than diabetes, breast cancer or myocardial infarction. Bone remodeling facilitates repair of microdamage and provides calcium from bone stores for cellular functions (Figure 1). The metabolic component of bone is made up of bone remodeling units (BRUs), over 1 million of which are active at any given time in a healthy adult woman. It is estimated that complete turnover of the skeleton occurs every 10 years. Bone remodeling is, however, accelerated in post-menopausal women, in whom estrogen deficiency results in increased bone turnover with an excess of resorption over formation. The fact that bone remodeling is an active and dynamic process enables the use of interventions in the treatment of osteoporosis that limit resorption (antiresorptive therapy) or augment formation (anabolic therapy).2

The activity of BRUs follows a well choreographed sequence of events. In the initial step, osteoclasts reabsorb bone over a period of about 3 weeks to create resorption cavities, which are collectively termed the remodeling space. Resorption is followed by osteoblast activation and formation of osteoid, which fills the resorption cavities over a period of about 3 months. When this active matrix synthesis is finished, osteoblasts become embedded in the matrix and function as osteocytes. These cells remain active in bone remodeling by maintaining connections to the bone surface, the BRUs and other osteocytes via an extensive canalicular network. Fluid flows through this network and is thought to induce signaling, thus permitting osteocytes to function as mechanoreceptors. Osteocytes are able to direct remodeling to areas that require repair.3

SUMMARY

Osteoporosis is a worldwide health problem with a high prevalence. Agents for the treatment of osteoporosis are classified as either antiresorptive or anabolic. Antiresorptive agents work by inhibiting the activity of osteoclasts and, therefore, reducing bone resorption. Currently available antiresorptive agents include bisphosphonates, selective estrogen-receptor modulators, calcitonin and estrogen. Various novel antiresorptive agents are in development. Receptor activator of nuclear factor κB ligand is an important cytokine involved in osteoclast activation; denosumab, a fully human monoclonal antibody to this molecule, has finished a major fracture trial. Assessment is underway of odanacatib—an inhibitor of cathepsin K, which is an osteoclast enzyme required for resorption of bone matrix. Glucagon-like peptide 2 is being evaluated for the prevention of the nocturnal rise in bone resorption without affecting bone formation. Anabolic agents act by stimulating formation of new bone. The only anabolic agent currently available in the US is teriparatide—recombinant human parathyroid hormone (PTH)1–34—and recombinant human PTH1–84 is available in Europe. PTH stimulates osteoblast function and bone formation. Novel anabolic agents in development include: antibodies such as sclerostin and dickkopf-1 that target molecules involved in Wnt signaling, a pathway that regulates gene transcription of proteins that are important for osteoblast function; an antagonist to the calcium-sensing receptor; and an activin receptor fusion protein, which functions as an activin antagonist and has shown promise as an anabolic agent in early human trials.

KEYWORDS anabolic agents, antiresorptive agents, calcium-sensing receptor, osteoporosis, Wnt signaling

REVIEW CRITERIA

A review of the literature was performed by searching PubMed using the following search terms "antiresorptive agents", "anabolic agents", "Wnt signaling", "calcium sensing receptor" and "novel osteoporosis therapies". Abstracts and full-text papers published between 2000 and 2008 were reviewed.
The therapies currently available to modify bone remodeling and, therefore, treat osteoporosis all have limitations. Treatment with antiresorptive agents, for example, eventually leads to a decrease in osteoblast function. The initial increase in bone mass that results from the use of this type of therapy is due to inhibition of bone resorption by osteoclasts, while osteoblasts continue to function and fill in the remodeling space. This uncoupling of formation and resorption, however, lasts for only a short time (about 2 years) before osteoblast function decreases and accumulation of bone mass begins to slow. Increases in bone mass after this time point are largely related to an increase in mineralization density—a result of reduced bone turnover. Bisphosphonates—a type of antiresorptive agent—have a long half life in bone; after discontinuation of therapy a residual amount of drug remains in bone and is available to osteoclasts in the future when further BMUs are activated. In rare cases, long-term therapy might lead to turnover being inadequate to repair microdamage, at which point bone is referred to as being adynamic. This low turnover state is thought to result in the accumulation and coalescence of microcracks, which results in fractures. For the anabolic agent teriparatide, use is limited to 24 months in the US and 18 months in Europe, as the clinical trial of this agent was discontinued after a mean treatment duration of 19 months because an animal toxicology study showed an increased incidence of osteosarcoma. Evidence from more than 6 years of clinical use, however, indicates that the development of osteosarcoma does not seem to be increased with the use of teriparatide.

Research is currently focusing on drugs that target the remodeling cycle by affecting osteoclasts, osteoblasts and osteocytes, and/or molecules that control signaling pathways important for cell function and gene transcription. The goal of this article is to review the types and modes of action of therapies that are currently available and those that are in development for the treatment of patients with low bone mass.

ANTITROMPTIVE AGENTS

Bisphosphonates are the most frequently used type of antiresorptive agent. These drugs bind avidly to hydroxyapatite and work by inhibiting farnesyl pyrophosphate synthase, an enzyme in the mevalonate pathway (Box 1). This pathway is important for protein prenylation—the attachment of lipids to proteins—which is critical for cytoskeletal organization in osteoclasts. Inhibition of the mevalonate pathway disrupts cytoskeletal structure, which prevents osteoclasts from forming a ruffled border on the bone during the remodeling process, generating a proton gradient, and resorbing minerals and matrix.

Bone resorption and formation are tightly coupled. As described above, inhibition of resorption eventually results in inhibition of formation. An agent that inhibits bone resorption but allows bone formation to continue would, therefore, have a greater effect on bone mass and quality than currently available agents. Mice that lack the chloride channel CLC-7 have no
functional osteoclasts and thus have reduced bone resorption, but do have ongoing bone formation. This finding suggests that the development of an agent that inhibits bone resorption but permits bone formation could be possible.8

**Box 1** Mechanism of action of nitrogen-containing bisphosphonates.

Nitrogen-containing bisphosphonates—such as risedronate sodium, zoledronic acid, disodium pamidronate, alendronic acid and ibandronic acid—work predominantly by inhibiting the enzyme FPPS, which catalyzes a step in the mevalonate pathway and inhibits protein prenylation. Protein prenylation involves the transfer of a farnesyl or geranylgeranyl isoprenoid lipid group onto the C-terminus of small GTPases. The GTPases are critical for osteoclast proliferation, apoptosis, membrane ruffling and membrane trafficking. Loss of these prenylated proteins results in loss of osteoclast function. Abbreviations: HMG-CoA, hydroxy-3-methylglutaryl coenzyme A; FPP, farnesyl pyrophosphate; FPPS, farnesyl pyrophosphate synthase; GGPP, geranylgeranyl diphosphate; GTP, guanine triphosphate.

**Glucagon-like peptide 2**

Glucagon-like peptide 2 (GLP-2) is a polypeptide hormone released from the intestinal mucosa in response to food intake. Bone remodeling occurs according to a circadian rhythm, with bone resorption rising in the night. The rhythm is affected by the rate of food intake, for example by nocturnal fasting. Treatment with GLP-2 at bedtime results in a substantial reduction in the bone resorption that normally occurs overnight. GLP-2 does not seem to reduce bone formation, as evidenced by stable levels of osteocalcin—a marker of bone formation—during treatment.10,11 A 120-day phase II trial of GLP-2 in 160 postmenopausal women demonstrated an increase in hip bone mineral density (BMD) and a reduction in the nocturnal rise in the concentration of C-telopeptide—a marker of bone resorption—with no effect on osteocalcin.10,11 If this pattern could be sustained in the long term, GLP-2 would have an advantage over currently available antiresorptive agents, which decrease bone formation.

**Cathepsin K inhibitors**

Cathepsin K is a cysteine protease that is selectively expressed by osteoclasts and can degrade key bone matrix proteins, including collagen. Elimination of cathepsin K in osteoclasts results in inhibition of bone resorption. Inhibitors of cathepsin K are suggested to have less of an effect on osteoclast–osteoblast interaction than available bisphosphonate antiresorptive agents, resulting in less inhibition of bone formation.

The first demonstration of the effect of cathepsin K inhibitors on BMD in humans was in a 12-month trial of balicatib in 675 postmenopausal women with BMD T scores of less than –2.0.13 In this study, markers of bone resorption declined by 55–61%, with no decline in markers of bone formation. BMD in the lumbar spine increased by 4.4% and in the hip by 2.2%. Skin reactions, including pruritis and morphea-like changes, were noted in a small number of patients. In addition, balicatib increased intact parathyroid hormone (PTH) levels by 50% in a small Japanese trial.14

Owing to adverse effects, especially skin reactions, drug development of all cathepsin K inhibitors except for odanacatib (formerly MK-0822) has been suspended. Cathepsin inhibitors other than odanacatib seem less specific for cathepsin K, which may account for the apparent differences in toxicity. Inhibition of cathepsin K in humans by orally bioavailable odanacatib is being evaluated in several ongoing trials. The 24-month results of a randomized controlled trial evaluating four doses of odanacatib given as a daily oral dose showed increases in spine and hip density (5.5% and 3.2%, respectively). Urine N-telopeptide of type I collagen (uNTX) and bone-specific alkaline phosphatase (BSAP) decreased 52% and 13%, respectively, in patients on odanacatib, whereas uNTX decreased by 5% and BSAP increased by 3% with placebo. These findings suggest that odanacatib produces less inhibition of bone formation than seen with current antiresorptive therapies.15
Denosumab
Receptor activator of nuclear factor κ B ligand (RANKL), a member of the TNF receptor family, is an important mediator of bone remodeling and is expressed by various cell types, including osteoblasts, synovial fibroblasts and activated T cells. RANKL binds to receptor activator of nuclear factor κ B (RANK) on osteoclast membranes and induces differentiation, activation and survival of these cells.16,17 A regulator of RANK–RANKL interaction is the soluble cytokine osteoprotegerin, which is a naturally occurring member of the TNF receptor family that acts as a decoy receptor by competing with RANKL for the bind sites of RANK (Figure 2). Denosumab is a human monoclonal IgG2 antibody that binds selectively and with high affinity to RANKL and pharmacologically mimics the effect of osteoprotegerin on RANKL. The results of a phase II randomized dose-ranging trial evaluating denosumab have been reported.18 At 24 months, increases in lumbar spine density ranging from 4.1% to 8.9% were observed. BMD gains at cortical sites, such as the hip and forearm, were greater with denosumab than with alendronic acid. No patient developed neutralizing antibodies during the trial. A 3-year phase III fracture trial examining denosumab administered subcutaneously in 60 mg doses every 6 months demonstrated 68%, 41% and 20% reductions in vertebral, hip and nonvertebral fractures, respectively, compared with placebo.19

Denosumab differs from bisphosphonates in several ways. First, levels of bone turnover markers nadir more rapidly, within a few days of injection, after denosumab treatment than following bisphosphonate therapy; after discontinuation, bone markers recover to normal levels more rapidly than with oral bisphosphonates. Second, there is no accumulation of denosumab in the bone, as there is with bisphosphonates. A third potential difference is that combining denosumab with PTH therapy could, in theory, have an additive effect on BMD. This effect is seen in animal models when osteoprotegerin is added to PTH therapy and is in contrast to the effect seen with the addition of bisphosphonates, which blunts the PTH response.

ANABOLIC AGENTS
Anabolic agents have the capacity to increase bone mass to a greater degree than antiresorptive agents. Not only are anabolic agents able to increase bone mass, but they also have the capacity to improve bone quality and increase bone strength—in part by affecting microarchitectural features such as connectivity, density and geometric features. These changes in quality cannot, however, be detected by current clinical measures of drug response, such as assessment...
of levels of bone turnover markers and dual X-ray absorptiometry. When quantitative CT, a volumetric measure of bone mass, is used to measure change in BMD during PTH treatment, the increases observed are substantially greater than those seen with dual X-ray absorptiometry, an area measure of bone mass. The use of quantitative CT to measure drug response in clinical practice is, however, prevented by the cost of this technique and the concern regarding the intensity of radiation required.

Parathyroid hormone

The recombinant human PTH1–34 teriparatide is the only anabolic agent currently available in the US for the treatment of patients with low bone mass, and recombinant human PTH1–84 is available in Europe.20 The mechanism of action of recombinant human PTH is still under investigation, but the drug probably affects multiple pathways and alters the activity of osteoblasts, bone lining cells, osteoclasts and osteocytes. PTH stimulates bone formation by increasing the number of osteoblasts, partly by delaying osteoblast apoptosis.21 Transient exposure to this hormone, by injection of a short-half-life (1–3 h) preparation, results in distinct anabolic effects. Continuous elevation of PTH levels, as seen in patients with hyperparathyroidism, usually results in catabolic effects and bone loss. The effects of PTH are mediated by a G-protein-coupled receptor, PTH receptor 1.

Different amino-terminal fragments of PTH, such as PTH1–31, could have a different anabolic effect to that of PTH1–34. Cyclic PTH1–31 has been hypothesized to produce a more anabolic profile than PTH1–34 or PTH1–84 because the PTH1–31 doses that provide a robust bone-formation response decrease bone resorption. A 12-month phase II dose-ranging trial in postmenopausal women demonstrated an increase in lumbar spine BMD of 11%.22 Selected amino acid substitutions at various positions in PTH1–28 have been shown to increase the activity of this hormone, suggesting that more potent PTH ligands might be available.23 Given that the duration of PTH treatment is limited to 2 years in the US and 18 months in Europe, there is an unmet need for additional anabolic agents.

Modulators of calcium-sensing receptors

The calcium-sensing receptor is a G-protein-coupled, seven-pass transmembrane molecule present in the parathyroid gland (and kidney), whose function is to coordinate calcium homeostasis by regulating the release of PTH.24 Manipulation of this receptor by small-molecule allosteric modulators can affect PTH secretion. Positive allosteric modulators—calcium-sensing-receptor agonists termed calcimimetics, such as cinacalcet—are used to lessen PTH secretion in patients with renal disease and hyperparathyroidism. Negative allosteric modulators—calcium-sensing-receptor antagonists termed calcilytics—are in development for the treatment of osteoporosis. These agents block the function of calcium-sensing receptors, resulting in a PTH pulse with each dose (Figure 3). Calcilytics can be administered orally and would, therefore, remove the need for daily injections associated with teriparatide administration.

Calcilytics must meet several important requirements before they can be useful as anabolic agents. First, they must stimulate the release of sufficient PTH. Second, anabolic action requires a short half-life and transient activation of the receptor, since in the case of calcilytics sustained activation would result in prolonged PTH secretion and a catabolic state, such as hyperparathyroidism. Third, the molecule should not exhaust the parathyroid gland, which would result in hyperplasia. A proof of concept study in rats with the calcilytic ronacaleret has shown that this PTH agent has a short half-life and produces a robust PTH response, increases both cortical and trabecular bone formation,
and generates notable increases in markers of osteoblast function—such as propeptide of type I procollagen, osteocalcin and bone-specific alkaline phosphatase—equivalent to those seen with teriparatide. A dose-ranging clinical trial of ronacaleret in humans was discontinued, however, due to a poor effect on BMD.

Modulation of Wnt signaling

Wnt proteins form a large family of extracellular cysteine-rich glycoproteins that help regulate embryogenetic bone remodeling and are involved in many additional cellular processes. Wnt proteins activate an intracellular pathway that results in accumulation of beta-catenin. Wnt permits association of the membrane receptors frizzled and lipoprotein-receptor-related protein 5/6 (LRP5/6) and activation of a protein complex consisting of axin, adenomatous polyposis coli and glycogen synthase kinase 3, which activates an intracellular pathway (Figure 4). In the absence of Wnt, glycogen synthase kinase 3 phosphorylates beta-catenin, which is then degraded via the ubiquitin–proteosome pathway. In the presence of Wnt, the protein complex is disrupted and phosphorylation of beta-catenin does not occur, so beta-catenin accumulates, translocates to the cell nucleus and binds to transcription factors that affect transcription of Wnt-responsive genes, which are important in bone formation.

Inhibitors of Wnt signaling can bind to frizzled (serum frizzled-related proteins), Wnt (Wnt inhibitory factors) or LRP5/6 (sclerostin and dickkopf-1). These agonist proteins prevent Wnt from activating the signaling pathway mediated by frizzled and LRP5/6 receptor, leading to a decrease in signaling and a consequent reduction in bone formation. By contrast, deficiencies in these inhibitors or antibodies result in increased Wnt signaling and, therefore, increased bone formation.

Sclerosteosis, a human disease of high bone mass, is the result of a homozygous mutation in the SOST gene, which encodes sclerostin. A deficiency of sclerostin results in increased Wnt signaling and high bone mass, and, in the skull, causes entrapment of cranial nerves and increased intracranial pressure, which can subsequently lead to stroke. Heterozygous mutations in the SOST gene result in moderate increases in bone mass and fewer skeletal complications.

Figure 4 Simplified view of Wnt and beta-catenin signaling. (A) Without Wnt, the scaffolding protein axin assembles a protein complex, beta-catenin is phosphorylated, ubiquitinated and degraded by the proteosome. (B) With Wnt, beta-catenin is not phosphorylated, and is instead translocated to the nucleus where it binds to the TCF transcription factor, activating Wnt-responsive genes. Two membrane proteins, Fz and LRP5/6, can associate in the presence of Wnt, which leads to formation of the protein scaffolding complex, accumulation or degradation of beta-catenin, and gene transduction. Abbreviations: Apc, antigen presenting cell; beta-Cat, beta-catenin; beta-TRCP, transducin repeat-containing protein; Ck1, casein kinase 1; Dvl, disheveled; Fz, frizzled protein; GsK3, glycogen synthase kinase 3; LRP, lipoprotein-receptor-related protein; TCF, T-cell factor. Reproduced with permission of the Company of Biologists.
Given that sclerostin is almost exclusively a product of osteocytes, the development of antibodies to this protein offers a way to specifically target bone formation. Antibodies to sclerostin increase bone formation in osteopenic estrogen-deficient rats. In postmenopausal women, a single subcutaneous dose of an antibody to sclerostin resulted in a 60–100% increase in the levels of propeptide of type I procollagen at day 84 of treatment, no increase in serum C-telopeptides, and a 6% increase in lumbar spine BMD.

Gain of function mutations in the gene encoding LRP5/6 lead to increased bone mass. These mutations impair binding of dickkopf-1 to LRP5/6 and permit increased Wnt signaling and bone formation. Antibodies against dickkopf-1 prevent binding of dickkopf-1 to LRP5/6 and increase bone mass, volume and formation in rodents. Antibodies to dickkopf-1 could be used as an anabolic agent for the treatment of patients with low bone mass.

**Activin inhibitors**

A key signaling component in bone formation is bone morphogenic protein (BMP), a member of the transforming growth factor β superfamily. BMPs signal via SMADs, which are nuclear transcription factors that regulate the activity of transforming growth factor beta ligands and specific genes involved in osteogenesis. Fibrodyplasia ossificans progressive—a genetic disorder of progressive heterotopic ossification—is caused by missense mutations in activin receptor IA, a BMP type I receptor, and leads to increased BMP signaling. This signaling promotes osteoblast maturation and function by binding to two distinct activin receptors—I and II—on the cell membrane. Activin binds to activin receptor IIA and is a negative regulator of bone mass, acting as an essential cofactor for osteoclastogenesis.

A fusion protein comprising the extracellular domain of human activin receptor IIA and the Fc portion of human IgG1 (ACE-011) has the capacity to bind to activin and prevent receptor binding. This antibody has been shown to increase bone mass in cynomologus monkeys (>70% increase in trabecular bone mass, as determined by quantitative CT). Administration of a single dose of the fusion protein to 48 postmenopausal women resulted in an increase in levels of bone-specific alkaline phosphatase and a decrease in C-telopeptides. The BMP pathway is a promising new area to target therapeutic agents for the treatment of low bone mass.

**CONCLUSIONS**

Notable advances in the treatment of low bone mass seem to be on the horizon and are a result of increased understanding of the mechanisms underlying bone formation and resorption. New anabolic agents affecting the calcium-sensing receptor and Wnt signaling, and new antiresorptive agents that might have less of an effect on bone formation than currently available therapies, offer promise for the treatment of low bone mass. Additional therapies, especially those that can be used to treat patients with established fractures, are needed to reduce the burden of osteoporosis.

**KEY POINTS**

- Drugs that influence bone mass do so by acting directly on cells that are integral to the bone remodeling unit
- Antiresorptive agents inhibit osteoclasts and prevent bone resorption
- Anabolic agents stimulate osteoblasts and, therefore, bone formation
- Potential new therapeutic agents include denosumab, an antibody to receptor activator of nuclear factor κ B ligand, and odanacatib, a cathepsin K inhibitor
- Potential new anabolic agents include those that target the calcium-sensing receptor and proteins in the Wnt signaling pathway

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Competing interests
C Deal has declared associations with the following companies: Amgen, GSK, Lilly, Novartis, Proctor & Gamble and Roche. See the article online for full details of the relationships.