# Anabolic Therapies

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**Abstract** The striking clinical benefits of intermittent parathyroid hormone in osteoporosis have begun a new era of skeletal anabolic agents. Recombinant human parathyroid hormone (rhPTH) (1–34) is the first US Food and Drug Administration–approved anabolic therapy. Its use has been limited by the need for subcutaneous injection. Newer delivery systems include transdermal and oral preparations. Newer anabolic therapies include monoclonal antibody to sclerostin, a potent inhibitor of osteoblastogenesis; and use of bone morphogenetic proteins and parathyroid hormone–related protein PTHrP, a calcium-regulating hormone similar to PTH.

**Keywords** Wnt · Sclerostin · BMP · PTH · BMD · Osteoporosis

## Introduction

The Wnt signaling pathway demonstrates a complex network of proteins well known for their roles in embryogenesis but also involving normal physiologic processes of bone formation in response to loading and unloading. The interaction between Wnt proteins and cell surface receptors can result in a variety of intracellular responses [1]. Studies have revealed the existence of extensive crosstalk between numerous ligands, receptors, and coreceptors, as well as between downstream intracellular messengers [1]. The Wnt pathway involves a large network of proteins that can regulate the production of Wnt signaling molecules and interaction with receptors and target cells, and the physiologic response of target cells that result in exposure of cells to the extracellular Wnt ligands. The canonical Wnt pathway describes events when Wnt proteins bind to cell surface receptors in the Frizzled family, causing the receptors to activate disheveled family proteins and ultimately result in the change in the amount of β-catenin that reaches the nucleus [1]. Several proteins have been described that inhibit Wnt signaling [2]. One such protein is sclerostin, which binds low-density lipoprotein receptor-related protein (LRP) and inhibits Wnt signaling. Antibodies to dickkopf and secreted frizzled-related protein produce similar outcomes in animal models to sclerostin [2]. This article discusses several anabolic agents, including sclerostin, bone morphogenetic protein (BMP), and parathyroid hormone (PTH), and new delivery systems for PTH.

## Sclerostin

Sclerostin, a glycoprotein secreted by osteocytes and to a lesser extent other cell types (vascular, kidney), is a potent inhibitor of osteoblastogenesis. Sclerostin is secreted from osteocytes and travels through the canaliculi to the bone surface where it binds to coreceptors LRP5 and LRP6 and prevents colocalization with frizzled protein and Wnt signaling, thereby reducing osteoblastogenesis and bone formation [3]. Loss-of-function mutations in *SOST* are associated with an autosomal-recessive disorder, sclerosteosis, which causes progressive bone overgrowth [4]. A deletion downstream of the *SOST* gene, which results in reduced sclerostin expression, is associated with a milder form of the disease called van Buchem disease [5].

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Furthermore, *SOST*-null mice have a high bone mass phenotype [6].

The development of a monoclonal antibody to sclerostin that can be administered subcutaneously has allowed scientists to evaluate the effect of sclerostin blockade on bone metabolism and bone mass. Li et al. [7] treated estrogen-deficient osteopenic rats with biweekly subcutaneous treatment with 25 mg/kg of a monoclonal antibody to sclerostin for 5 weeks and restored trabecular bone mass to baseline levels. Surface-based histomorphometry determined that the increase in bone mass resulted from an increase in bone mass at all skeletal envelopes, including cancellous, cortical bone sites, and supervertebral sites. The increase in bone mass and change in microarchitecture were associated with improved bone strength in both the appendicular and axial skeleton.

Sclerostin (*SOST*) is thought to function as a paracrine inhibitor of canonical Wnt signaling on the preosteoblast surface by binding to the Wnt coreceptors LRP5 and LRP6 and blocking their colocalization with the frizzled receptor [8]. The bone-forming effects of the *SOST* antibody in many ways resemble those of high-dose intermittent PTH in rodents. Several studies have reported that sclerostin gene expression and protein levels are reduced in animals treated with daily injections of human parathyroid hormone (hPTH) (1–34) [9]. Preclinical studies with a sclerostin inhibitor appear to be somewhat different from those with hPTH (1–34). For example, all skeletal sites respond to anabolic daily PTH treatment; the trabecular bone is most responsive, followed by the endosteal surface and the periosteal surface. In contrast, inhibition of sclerostin also results in significant bone formation at the periosteal surface. Also, studies find the increases in bone formation induced by antisclerostin antibody, unlike PTH, are not associated with increases in bone resorption in the aged rodent skeleton.

Reduced mechanical stimulation leads to disuse osteoporosis, as seen in bedridden patients and in astronauts. Lin et al. recently [10] reported that *SOST* knockout mice were resistant to mechanical unloading bone loss. In contrast to wild-type mice [9], Wnt/β-catenin signaling was not altered by unloading in *SOST* knockout mice. The data suggest a potential major role for sclerostin in mediating the bone response to unloading and propose it may be a promising target for preventing disuse osteoporosis [9].

At this time, the monoclonal antibody to sclerostin in is early phase 2 clinical trials in postmenopausal women with osteoporosis and in fracture healing studies, leaving only phase 1 clinical safety data and short-term preclinical studies. The long-term safety of sclerostin has not been addressed. Additional clinical study data are needed to determine the rapid gain in bone mass is associated with bone of normal strength and architecture, if boney overgrowth occurs at areas such as the carpal tunnel resulting in carpal tunnel syndrome, or around the lumbar spine neural foramen resulting in lumbar radiculopathy or spinal stenosis. In summary, treatments based on inhibition of sclerostin may be a powerful way to restore bone strength of the osteoporotic skeleton in our patients and potentially to provide more efficacious protection from hip fracture than current therapies.

## Bone Morphogenetic Proteins

BMPs are members of the transforming growth factor-β superfamily. Bone morphogenic proteins are being used successfully clinically for treating spinal fusion, delayed fracture healing, and nonunion [10]. Multiple in vitro and in vivo studies and clinical trials show a strong effect of BMP-2 and BMP-7 in terms of inducing bone [11–13]. Clinical application is currently restricted in part due to cost. BMP-2 is used for open tibial fractures and spinal fusions, whereas BMP-7 is used for nonunion with limited indication for spinal fusion [14]. BMPs appear to be as good as iliac crest bone graft or even more effective with moderate side effects [10].

There is a need for anabolic agents such as BMP for patients with delayed fracture healing or nonunion. A literature review on tibial fracture healing reveals that 16.7% of patients showed delayed fracture healing or a nonunion, and 11.8% developed malunion with up to 23% requiring operative reintervention [15]. Successful bone healing depends on two factors: 1) mechanical signaling [16], which can be enhanced by biophysical stimulation (eg, ultrasound, shockwaves, and electromagnetic fields), or 2) biological substances (eg, bone grafts, hormones, and growth factors such as BMP) [17].

BMPs appear to be most beneficial for atrophic nonunions, but may also be used for treating hypertrophic nonunions. Treatment of bone defects in nonunion often requires the use of additional grafting material, which may be autogenous bone or potentially, in the future, the use of stem cells. BMP-7 is often used in combination with synthetic carriers based on tricalcium phosphate. BMP-2 is approved for anterior lumbar interbody fusion, but also has shown beneficial results in transforaminal lumbar application in multiple clinical studies [17, 18]. In posterolateral lumbar fusion, the use of BMP-2 was compared with iliac crest bone graft and showed a significantly higher fusion rate compared with control subjects [19].

Side effects of BMPs are moderate [19–21]. For posterior fusion, heterotopic bone formation was found in the spinal canal close to the cage, raising concerns about safety aspects and that BMP should not be applied too close to the spinal canal. Concerns also have been raised with transforaminal lumbar interbody fusion in which initial vertebral resorption

or osteolysis was found, which resolved after 3 months. Although BMP-2 has been felt to be safe and effective for anterior cervical spine fusion, dysphagia has been noted, possibly related to transient postoperative soft tissue swelling, which usually disappears within a few days.

Preclinical and clinical safety assessments revealed little evidence of toxic effects and few reports of adverse effects such as ectopic bone formation, bone resorption, swelling, and hematoma. A low level of immunologic reaction has been seen with antibody responses detected in less than 1% of patients.

Use of BMPs, however, is limited by their expensive cost. Current use of BMPs requires an open approach; they are currently applied in combination with collagen. Possible new application strategies may include the local and controlled injection of BMPs in combination with a synthetic carrier, such as a calcium phosphate paste.

## New Delivery Systems in Development for PTH Treatments

Nearly 7 years ago, the US Food and Drug Administration approved the first anabolic agent, recombinant human parathyroid hormone (rhPTH) (1–34), the 34 amino acid fragment of PTH, to be given on a daily basis by subcutaneous injection for up to 2 years for treating postmenopausal osteoporosis [22]. PTH is anabolic because it increases bone formation and bone mass, but it also stimulates bone remodeling as part of its action. PTH increases bone formation through several actions, including increasing commitment of mesenchymal stem cells to the osteoblast lineage, increasing osteoblast maturation and possibly life span, and reducing the osteocyte production of sclerostin to further stimulate bone formation. PTH stimulation of osteoblastogenesis also increases receptor activator of nuclear factor-κB ligand production, which then stimulates osteoclast maturation and activity, increasing bone remodeling overall; however, the overall effect is a positive formation balance. Although rhPTH (1–34) is effective in improving bone strength and incident fracture risk reduction, since it must be administered by daily subcutaneous injection for up to 2 years, a number of osteoporotic patients who might benefit from rhPTH (1–34) treatment do not choose to be treated [23].

Because PTH (1–34) is a peptide hormone, many potential challenges must be overcome to administer it orally. In an attempt to develop a simpler mode of delivery for PTH (1–34), a PTH (1–34) that is delivered by a transdermal microneedle delivery system patch has been developed and now tested in phase 1 and phase 2 clinical trials. The PTH transdermal patch is composed of a small adhesive patch that is coated with hPTH (1–34) on a titanium microneedle array with 1,300 microneedles per hPTH (1–34) patch [24, 25••, 26, 27]. A recent phase 2 clinical trial was completed that evaluated the effectiveness of the transdermal hPTH (1–34) compared with rhPTH (1–34) subcutaneous injections and a placebo patch on bone mineral density (BMD) in postmenopausal women with osteoporosis after 6 months of treatment. A total of 165 patients were enrolled in the study and randomized to an hPTH (1–34) patch of 20 μg, 30 μg, 40 μg, or placebo, or rhPTH (1–34) injections for 6 months. After 6 months of therapy, subjects treated with all doses of the hPTH (1–34) patch and the rhPTH (1–34) given subcutaneously had significant gains in lumbar spine BMD (range, ~3–5% gain) compared with the placebo patch (−0.02%). In addition, the average gain in total hip BMD in the hPTH (1–34) 40-μg dose group was significantly greater than the placebo group (~1.5% compared with -0.5%), with no significant change with the 20-μg or 30-μg dose. Changes in the biochemical markers of bone turnover were somewhat different between the two deliver systems for PTH. The rhPTH group had a gain in the bone formation marker, P1NP, of nearly 300% and a rise in the bone resorption marker, CTX-1, of nearly 100% over the baseline values. The transdermal patch hPTH (1–34) 40-μg/d group had gains in bone formation by P1NP of only 100%, and CTX-1 of only about 40% [24].

The explanation for the greater change in BMD of the lumbar spine and hip by the higher doses of the hPTH (1–34) group may be that less bone remodeling was stimulated; however; this is a hypothesis that will require additional studies. One other interesting observation was that the mean plasma concentrations of PTH (1–34) with transdermal delivery peaked after about 5–10 min, whereas the peak was nearly 30 min after the subcutaneous injection, and the serum half-life after the transdermal administration was shorter (~60 min), whereas it was about 90 min with subcutaneous administration [24]. The different pharmacokinetics between these two delivery systems may explain the changes we observe in bone turnover. However, phase 3 studies of longer duration and more study subjects are needed to elucidate these phase 2 study results.

## Oral Delivery of PTH

A new formulation of PTH (1–34) is being developed as an oral formulation as a way to make this treatment a more convenient option for osteoporotic patients. The oral formulation includes the PTH (1–34) protein and an absorption enhancer, 5-CNAC [28]. A phase 1 study enrolled 34 postmenopausal women and 16 subjects. The subjects were randomized to receive placebo, rhPTH (1–34), or 1, 2.5, 5, or 10 mg of oral PTH (1–34) formulated

with 200 mg of 5-CNAC, 16 patients were randomized to receive up to six regimens of rhPTH (1–34) or 2.5 or 5 mg of oral hPTH (1–34) with 100 or 200 mg of 5-CNAC, and treatments were administered for at least 6 days [28]. They reported all doses of the oral PTH were well absorbed and serum concentrations increased in a dose-dependent manner. The dose of PTH (1–34) of 2.5 or 5 mg combined with the oral enhancer CNAC at 200 mg had an area under the curve that was very similar to rhPTH (1–34) given by subcutaneous injection. The side effects of rhPTH (1–34) given by subcutaneous injections can be hypercalcemia; however; in this phase 1 study, ionized calcium remained within normal limits for all evaluated doses. Adverse events reported that resulted in study subjects withdrawing from the study were symptomatic hypotension ($n$=3 from the PTH [1-34] at 2.5 or 5 mg with 200-mg CNAC; $n$=1 rhPTH [1-34]; $n$=1 placebo), three withdrew from prolonged vomiting (5-mg PTH [1–34] and 200-mg CNAC), and symptomatic but unconfirmed hypercalcemia ($n$=1 from the PTH [1-34] at 2.5 mg with 100-mg CNAC) [28].

This novel oral delivery system for hPTH (1–34) combined with an absorption enhancer CNAC appears to show promise for treating osteoporosis. Additional studies and refinement of the dosing regimen appear warranted to enhance the use of this novel technology.

## Parathyroid Hormone–Related Protein

Parathyroid hormone–related protein (PTHrP) is a hormone that is closely related to another hormone discovered in the 1920s named *parathyroid hormone* (PTH). Similar to PTH, PTHrP is also a hormone that regulates calcium metabolism. PTH has been shown to be effective in treating osteoporosis in animals and humans [29]. PTHrP has been shown to be effective in treating osteoporosis in laboratory animals, and there are strong scientific reasons to think that it may be effective in humans also [30]. A study of PTHrP (1–36) was performed to determine if it could increase bone mass in postmenopausal women with osteoporosis when administered daily by subcutaneous injection for 3 months [31].

The double-blind, placebo-controlled, randomized clinical study enrolled 16 postmenopausal women with osteoporosis between 50 and 75 years of age [31]. All had been on hormone replacement therapy for an average of 8 years and still had osteoporosis. Women who had been taking any other type of osteoporosis medication were excluded from the study. Half of the participants received a self-administered injection of PTHrP and the other half received a placebo. The patients were followed up for 3 months and the participants tolerated the treatment without developing hypercalcemia, hypotension, nausea, flushing, or other adverse effects. The lumbar spine BMD increased by

4.7% during the 3-month treatment period, a larger increase in bone gain that is usually observed with the available antiresorptive osteoporosis medications. Even though the study was designed as a pilot, the results were surprisingly more favorable than expected, and larger studies of longer duration are needed to elucidate the full potential of this bone-building agent and if the increase in bone mass can reduce incident fracture risk.

## Conclusions

We now have a diverse menu of osteoporosis therapies including both antiresorptive and one anabolic therapy (teriparatide). Current research suggests that in the future we may have multiple different anabolic therapies such as sclerostin, PTHrP, and other potential molecules, and alternate delivery systems for administration. The therapies may have orthopedic benefits in terms of fracture healing and fusions. The future of anabolic therapies looks bright.

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