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Review

Handling Parathormone Receptor Type 1 in Skeletal Diseases: Realities and Expectations of Abaloparatide

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Musculoskeletal disorders represent an elevated socioeconomic burden for developed aging societies. Osteoporosis (OP) has been treated with antiresorptive therapies or with teriparatide that was until recently the only anabolic therapy. However, approval of osteoporosis treatment in postmenopausal women with abaloparatide, which is an analog of parathyroid hormone-related peptide (PTHrP), has created a new alternative for OP management. The success of this new treatment is related to differential mechanisms of activation of PTH receptor type 1 (PTH1R) by abaloparatide and PTH. Here, we address the distinguishing mechanisms of PTH1R activation; the effects of PTH1R stimulation in osteoblast, osteocytes, and chondrocytes; the differences between PTH and abaloparatide actions on PTH1R; potential safety concerns; and future perspectives about abaloparatide use in other musculoskeletal disorders.

Abaloparatide, a New Strategy to Treat Osteoporosis

Musculoskeletal disorders are a major health problem and a socioeconomic burden in developed countries. In particular, **osteoporosis (OP)** (see Glossary) and **osteoarthritis (OA)** have a higher prevalence in aging populations and their incidence is increasing, reaching numbers of a global epidemic [1]. These facts made the World Health Organization (WHO) declare the past decade (2000–2010) as the Bone and Joint Decade [2] to raise the attention of health professionals and political authorities about this issue. It has been estimated that 200 million people worldwide suffer from OP [1], with around 50 million cases in the USA [3] and another 27 million in the EU [4]. Moreover, the number of fractures related to OP are increasing and they are also associated with an increase in morbidity and mortality [5]. In 2000 it was estimated that there were 9 million OP-related fractures per year worldwide [6]. In 2010 in the EU it was reported that 3.7 million fractures where related to OP with an associated cost of €37 billion [4] whereas it was estimated that in 2008 in the USA this cost was around $22 billion [7]. Furthermore, a recent study has reported that the average cost per fracture in osteoporitic patients in the USA from 2010 to 2013 was $12 839 [8].

OP is the most prevalent disease affecting bone tissue and is defined as a metabolic bone disease characterized by low **bone mass**, a deterioration in bone tissue microarchitecture, and an increase in the susceptibility to fractures [9]. There are several therapies to treat OP, which are categorized into two main groups: **antiresorptive** and **anabolic**. Among antiresorptive therapies, bisphosphonates are the most commonly used. These are compounds with a high affinity for bone hydroxyapatite that can inhibit osteoclast (Box 1) differentiation and function, and induce apoptosis [10]. Indeed, bisphosphonates have proven efficacy reducing fracture incidence [11]. Denosumab is a humanized monoclonal antibody against the **receptor activator of nuclear factor κB ligand (RANK-L)** that exerts a potent antiresorptive action in bone [12]. RANK-L is needed for the formation and activation of **osteoclast** cells and is mainly produced by cells of the **osteoblast** lineage [13]. Denosumab binds to RANK-L, impairing its interaction with the receptor RANK in the osteoclast precursor, inhibiting osteoclast differentiation and activation [12]. Other not so widespread antiresorptive strategies are the selective estrogen receptor modulators (SERMs), such as raloxifene, or hormone replacement therapy [14]. However, it has been reported that treatment for long periods with antiresorptive therapies leads to a lack of bone turnover, which is needed to replace aging or defective bone [15]. Among anabolic drugs the only available treatment for years was **teriparatide**, a peptide that is structurally identical to portion 1–34 of human **parathyroid hormone (PTH)**. Teriparatide binds to PTH receptor

Highlights

Management of musculoskeletal disorders such as osteoporosis and osteoarthritis are a major challenge for developed countries associated with a high socioeconomic burden given the high prevalence of these diseases and the aging population.

Treatment of osteoporosis has been addressed by anabolic or antiresorptive therapies.

Abaloparatide, a peptide that signals through the parathormone 1 receptor (PTH1R) has been recently added to the armamentarium of bone anabolic therapies.

Selective activation of PTH1R is the key of abaloparatide success in decreasing risk of fracture and increasing bone mineral density in postmenopausal women.

Use of abaloparatide might be extended to other musculoskeletal disorders.

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Box 1. Bone and Cartilage Microenvironments

Bone Microenvironment

The dynamic nature of bone is a consequence of the coupled activities of bone microenvironment cells. Osteoblasts are bone cells with a single nucleus and an average lifespan of about 3 months that produce bone tissue by secretion and mineralization of bone matrix proteins. Osteoblast differentiation from osteogenic precursors and osteoblast actions are both locally and systemically controlled during bone modeling and remodeling during development and throughout life by multiple factors. For example, PTH peptides, bone morphogenetic proteins, and Wnt/β-catenin signaling trigger the upregulation of transcription factors like Runt-related transcription factor 2 (Runx-2) that are indispensable for osteoblast differentiation and function.

Osteoclasts are large and multinucleated cells with an average lifespan of 2 weeks that are involved in the breakdown and resorption of bones. Osteoclast formation through fusion of mononuclear precursor cells of the hematopoietic lineage depends on signaling through c-Fms (receptor for M-CSF) in mononuclear precursor cells, which upregulates expression of RANK. Signaling through c-Fms and RANK in mononuclear precursors is the key driver of osteoclast formation. Regulation of osteoclast lifespan by controlling programmed cell death (apoptosis) is also an important mechanism to control osteoclast number. Osteoclast apoptosis is regulated by signaling pathways that include extracellular signal-regulated kinase (ERK), the serine/threonine protein kinase AKT, and mammalian target of rapamycin (mTOR).

Osteocytes are terminally differentiated bone-matrix-encased cells of the osteoblastic lineage with long dendritic processes and a lifespan of years. They are the most abundant bone cell type, accounting for 95% of all bone cells. Osteocyte cells orchestrate the complex bone remodeling processes by coordinating the activities of both osteoclasts and osteoblasts. Osteocytes are the main producers of RANK-Ligand and sclerostin, an inhibitor of Wnt/β-catenin signaling.

Cartilage Microenviroment

Cartilage is a differentiated connective tissue that obtains nutrients by diffusion from adjacent tissues. Blood vessels, lymph vessels, and innervation are generally absent in this tissue.

The primary cell type in cartilage is the chondrocyte, which resides within extracellular matrix spaces known as lacunae and produces the cartilage matrix, composed of collagens, proteoglycans, and glycosaminoglycans. Chondrocytes differentiate from cells derived from cartilage mesenchymal stem cells known as chondroprogenitors. Chondrocyte differentiation is controlled at multiple steps by mutual regulation of two factors, PTHrP and Indian hedgehog.

Glossary

Abaloparatide: analog of PTHrP that increases significantly BMD and reduces fracture risk.
Anabolic (therapy): therapy with compounds that increase bone mass. Currently, parathyroid hormone (teriparatide), PTHrP (abaloparatide), and an antibody to sclerostin (romosozumab).
Antiresorptive therapy: therapy with compounds that are used to increase bone strength in individuals with OP. Antiresorptive compounds have high affinity for bone hydroxyapatite, can inhibit osteoclast differentiation and function, and induce osteoclast apoptosis (e.g., bisphosphonates).
β-Arrestins: family of proteins that play dual roles in PTH1R signaling regulation (increase or decrease different signaling pathways).
Biased agonism: ligand-dependent selectivity for certain signal transduction pathways in the same receptor.
Bone mass: total amount of bone tissue in the body.
Bone mineral density (BMD): amount of mineralized bone tissue in a given area.
Cartilage: specialized connective tissue that covers and protects the ends of long bones at the joints and is a structural component of several organs that require a shock-absorbing and elastic tissue.
Chondrocytes: cartilage cells that synthesize and maintain the cartilaginous matrix.
Endochondral ossification: endochondral ossification is one of the processes in the development of the skeletal system in which bone tissue is produced from the cartilaginous tissue.
Intramembranous ossification: intramembranous ossification is one of the processes in the development of the skeletal system in which bone tissue is produced from mesenchymal cells.
Ossification: process by which bone is formed.
Osteoarthritis (OA): degenerative disease in which the cartilage of the joints breaks down leading to pain, stiffness and swelling.
Osteoblasts: bone cells that synthesize bone matrix.

type I (PTH1R) in osteoblast cell lineage, inducing bone formation, although it is also known to indirectly stimulate osteoclastic cells and promote bone remodeling. In any case, teriparatide has a net anabolic effect, increasing **bone mineral density (BMD)** associated with a reduction in vertebral and nonvertebral fractures [16]. Romosozumab, a monoclonal antibody that binds and blocks the Wnt pathway inhibitor sclerostin, is another anabolic therapy approved by the US FDA for postmenopausal women with high risk of fractures [17]. Recently, an additional anabolic therapy, **abaloparatide**, was approved by the FDA, for treatment of **postmenopausal osteoporosis**. Abaloparatide is a 34-amino-acid synthetic analog of PTHrP with 10 different amino acid residues at the carboxy terminus compared to physiological PTHrP (1-36) that binds to PTH1R, significantly increasing BMD and thus reducing fracture risk, likely through different mechanisms than teriparatide, due to its distinct selectivity for PTH1R [18]. Remarkably, abaloparatide seems to cause less bone resorption than teriparatide [18].

Here, we examine the mechanisms of action, advantages and disadvantages, safety concerns compared with other antiosteoporotic treatments, and possible future applications and perspectives of the new anabolic treatment for OP in postmenopausal women, called abaloparatide.

Ligands of PTH1R: Two Site Model and Biased Agonism

PTH1R transmits stimuli by two different naturally occurring polypeptide ligands: the endocrine-acting PTH, which is secreted from the parathyroid glands and the paracrine- and intracrine-acting

PTHrP, which is secreted from a wide range of tissues. Although both PTH and PTHrP signal via the same receptor and have similar agonist profiles stimulating PTH1R-dependent signaling pathways, the biological functions of the two ligands are different. PTH acts on the bone and kidneys, regulating blood levels of calcium and phosphate, whereas PTHrP typically promotes cell proliferation and differentiation in developing tissues.

Fragments consisting of the first 34 amino acids of both PTHrP and PTH contain the key functional elements for receptor interaction [19]. Residues 15–34 of PTH and 12–34 of PTHrP are necessary for binding to the extracellular N-terminal domain of PTH1R (site 1). The N-terminal residues of PTH(1–14) and PTHrP(1–11) interact with the transmembrane and extracellular connecting loop domains of PTH1R (site 2), triggering conformational changes in the receptor that initiate coupling and activation of G proteins leading to stimulation of intracellular signaling pathways. Residues at position 2, 5, and 8 are specially relevant for binding to the receptor and induction of cAMP signaling by both peptides (PTH: Val, Ile, Met; PTHrP: Val, His, Leu) [20,21].

PTH(1–34), PTHrP(1–36), and abaloparatide have similar binding affinities to PTH1R but show **biased agonism** in that they differentially regulate cAMP-dependent signaling pathways (Figure 1) [22]. All of them trigger cAMP-dependent responses after similar periods of stimulation while achieving different durations and magnitudes of response [22,23]. Structurally, PTH1R peptide ligands bind to distinct receptor conformations and thereby induce signaling responses that differ in duration. Ligands that preferentially bind to a heterotrimeric G-protein-independent conformation (R^0) induce responses of prolonged duration, while ligands that bind to a heterotrimeric G-protein-dependent conformation (R^G), induce responses of short duration [24]. PTH(1–34), PTHrP(1–36), and abaloparatide maintain comparable affinities for the R^G state of PTH1R but abaloparatide exhibits the highest selectivity for the R^G conformation over the R^0 conformation when compared to PTHrP(1–36) and PTH(1–34) (Table 1) [23]. Thus, even though all PTH1R agonists trigger cAMP signaling with similar potencies, abaloparatide induces transient cAMP-dependent responses compared to PTH(1–34) or PTHrP(1–36), and therefore, induces comparatively reduced accumulation of intracellular cAMP levels [22,23]. Other studies suggest that PTH1R ligands follow different pathways of internalization, being those with higher affinity to R^0, such as PTH(1–34), able to sustain prolonged Gαs-cAMP signaling in internalized endosomal domains, whereas PTH1R ligands with higher affinity to R^G (PTHrP analogs) dissociate earlier from the receptor without signaling in endosomes [25].

Since abaloparatide elicits a shorter cAMP response it thus induces submaximal activation/expression of cAMP-dependent downstream effectors, such as protein kinase A (PKA), CREB, c-Fos, and RANK-L, compared to PTH(1–34) or to PTHrP(1–36) [22]. Even limited cAMP responses of abaloparatide have been described to be sufficient to induce similar inhibition of sclerostin – via PKA stimulation – to PTH or PTHrP responses, triggering comparable activation of the anabolic Wnt signaling pathway. By contrast, abaloparatide barely activates the osteoclastogenic signals c-Fos and RANK-L, given that these factors require prolonged cAMP responses [22]. Based on these findings, it has been proposed that the aforementioned PTH1R agonists have comparable osteoanabolic features whereas only abaloparatide elicits low resorptive actions [22]. Structural and signaling properties of PTH1R are described in Box 2.

PTH1R in Osteoblasts

Osteoblasts play an important role in bone homeostasis by depositing and mineralizing new bone matrix. PTH(1–34), PTHrP(1–36), and abaloparatide bind PTH1R in the osteoblast cell lineage and produce both anabolic and catabolic effects depending on the method of administration. The intermittent treatment (daily treatment) with PTH/teriparatide, PTHrP, and abaloparatide has anabolic effects on bone resulting in increased bone mass [26,27]. These actions on bone may be due to a PTH/PTHrP-dependent increase in the number of osteoblasts originated by promotion of osteoblast proliferation and/or differentiation after intermittent stimulation with the ligands. The response to PTH and PTHrP is largely dependent on the cell line type, species, and differentiation stage of osteoblasts. Previously published results have shown that PTHrP and PTH prevent apoptosis of immature preconfluent

Osteoclasts: bone cells that degrade (resorb) bone matrix.
Osteocytes: cells that transduce mechanical stimulus into signals that regulate osteoblast and osteoclast functions.
Osteoporosis (OP): metabolic bone disease characterized by low bone mass, a deterioration in bone tissue microarchitecture, and an increase in the susceptibility of fractures.
Parathyroid hormone: hormone that controls levels of calcium and phosphate in the blood and influences bone loss and growth.
Parathyroid hormone type 1 receptor (PTH1R): parathyroid hormone/parathyroid hormone-related protein receptor (PTH/PTHrP type 1 receptor) is a class B G-protein-coupled receptor that regulates skeletal development, bone turnover, and mineral ion homeostasis.
Postmenopausal osteoporosis: type of OP resulting from estrogen deficiency in women.
Receptor activator of nuclear factor κB ligand (RANK-L): molecule whose main function is the activation of osteoclasts (cells involved in bone resorption) by binding to RANK.
Receptor desensitization: series of mechanisms to protect cells from overstimulation of a receptor by a given ligand.
Teriparatide: peptide that is structurally identical to portion 1–34 of human PTH that binds PTH1R in osteoblastic cells, inducing bone formation.

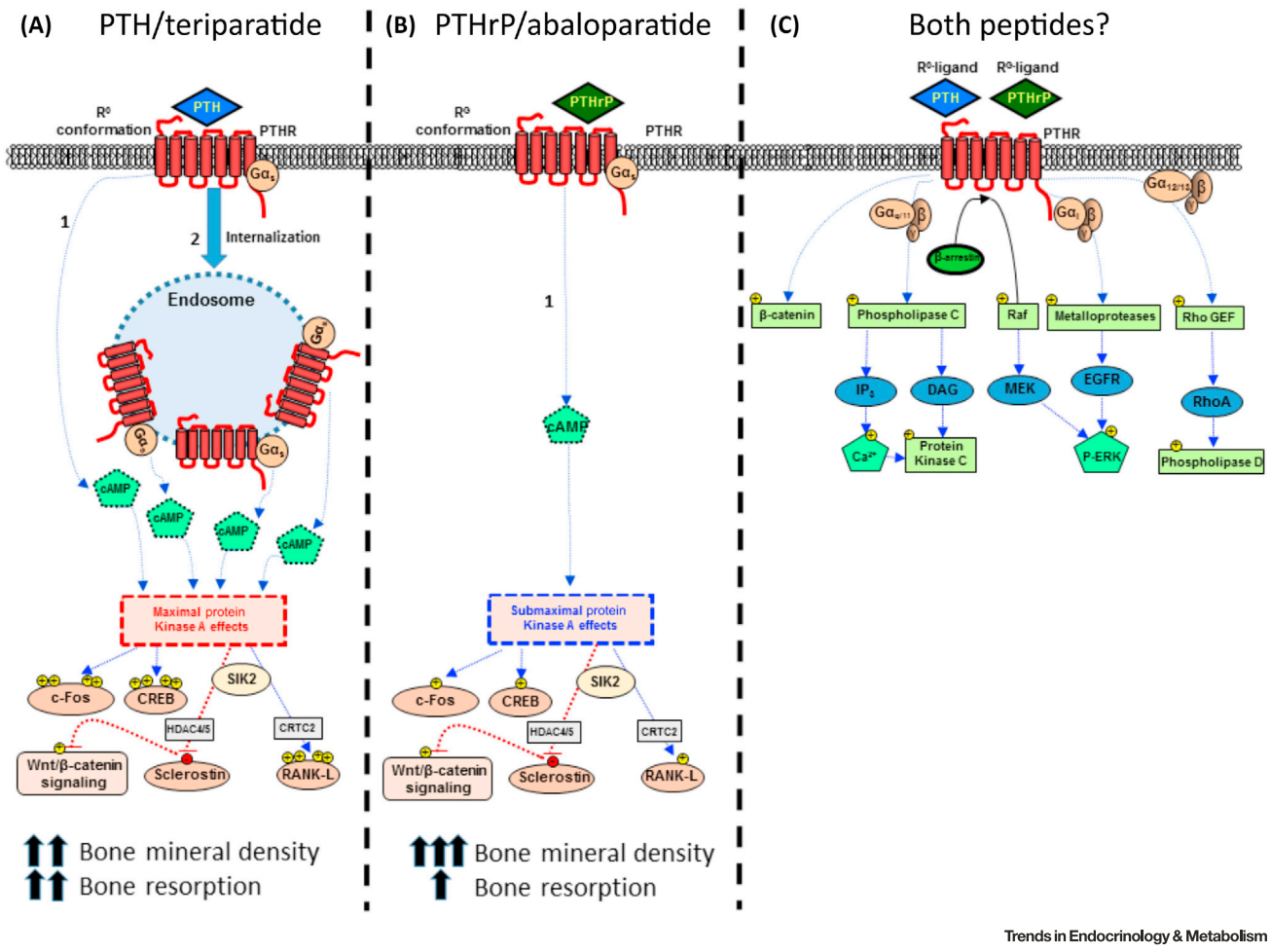

Figure 1. Model of Parathyroid Hormone (PTH)/Teriparatide and PTH-Related Peptide (PTHrP)/Abaloparatide Activation of Parathyroid Hormone Type 1 Receptor (PTH1R).

A proposed model of differential activation of PTH1R by PTH and PTHrP analogs is shown. (A) PTH/teriparatide induces generation of cAMP by a mechanism dependent on the activation of Gαs at the cell membrane (shown in Step 1) and by internalization of the receptor bound to Gαs in endosomes (shown in Step 2). As a result of both mechanisms (Steps 1 and 2) prolonged cAMP generation is induced and high concentrations of cAMP are produced, thus potentiating maximal protein kinase A (PKA) actions and expression of the PKA downstream effectors c-Fos, CREB and receptor activator of nuclear factor κB ligand (RANK-L) (via salt-inducible kinase 2; SIK2). By contrast, sclerostin in inhibited by a PKA/SIK2-dependent mechanism causing stimulation of the Wnt/β-catenin signaling pathway. These signaling pathways induce both increased bone mineral density and bone resorption. (B) PTHrP/abaloparatide induces generation of cAMP by a mechanism dependent on the activation of Gαs only at the cell membrane (shown in Step 1). As a result, a shorter cAMP response is induced leading to lower concentrations of cAMP and thus triggering submaximal PKA actions and expression of the PKA downstream effectors c-Fos, CREB, and RANK-L (via SIK2 pathway). Sclerostin is inhibited by submaximal PKA/SIK2-dependent actions causing stimulation of the Wnt/β-catenin signaling pathway (comparatively similar to PTH/Teriparatide). These signaling pathways induce increased bone mineral density and low bone resorption. (C) Five major intracellular signaling pathways that potentially could be selectively affected by PTH/teriparatide and PTHrP/abaloparatide are depicted: β-catenin; Gαq/11-phospholipase C-IP3/DAG-protein kinase C; β-arrestin–Raf–MEK–ERK; Gαi-metalloproteases–EGFR–ERK; and Gα12/13-Rho GEF–RhoA–Phospholipase D. Activation (+) or inhibition (–) of signaling pathways are shown with blue or red lines, respectively. Maximal effects are shown as multiple cross symbols (++++) and submaximal effects as a single cross symbol (+).

mesenchymal cells and enhances osteoblastogenesis, rather than adipogenesis [28,29]. PTHrP has also been shown to induce apoptosis of mature postconfluent cells [30]. Activation of apoptosis in mature osteoblast cells has been suggested as a potential mechanism to remove cells that no longer secrete matrix and thus, provide space for new matrix-producing cells. Furthermore, PTHrP is able to induce G1 phase growth arrest in differentiated osteoblasts targeting cyclin D1, CDK1 p21, p27, and

	Magnitude of the effect		
Effect	Abaloparatide	PTHrP	PTH
PTH1R R^G affinity	++	++	++
PTH1R R^0 affinity	+	++	+++
cAMP accumulation	+	++	+++
Duration of cAMP response	++	++	+++
PKA activation	+	++	+++
CREB activation	+	++	+++
c-Fos	++	++	+++
RANK-L expression	++	++	+++
Sclerostin inhibition	–	–	–

Table 1. Comparative Effects of Abaloparatide, PTHrP and PTH on PTH1R Signaling Pathways[a]

[a]Magnitude of the effect: +, low increase; ++, medium increase; +++ high increase; –, decrease.

p16 proteins [31]. These observations highlight the relevance of PTHrP in bone turnover and suggests that PTHrP plays a key role at multiple stages during bone development through its effects on cell survival [26]. In contrast to intermittent treatment, continuous administration of PTH or PTHrP triggers some catabolic effects on bone similarly promoting bone resorption-related factors such as RANK-L, RANK-L/OPG, and macrophage colony-stimulating factor (M-CSF) in osteoblasts [27,32]. Transient treatment with abaloparatide compared to teriparatide showed significantly attenuated expression of bone-resorption-related factors and M-CSF protein secretion [27].

Moreover, intermittent PTH induces RANK-L expression through salt-inducible kinases (SIKs) and nuclear translocation of cAMP-regulated transcriptional coactivator (CRTC) [33]. By contrast, the cAMP/PKA/SIK/CRTC2 signaling axis is weakly activated by PTHrP(1–36) and abaloparatide compared to PTH, and thus these peptides do not trigger as strong RANK-L responses as PTH does [22]. These observations could justify the differences in resorption induced by PTH(1–34), PTHrP(1–36), and abaloparatide. All of these peptides exert similar effects on the regulation of osteoblastic genes and on the suppression of the antianabolic protein sclerostin in osteoblast cells [22].

PTH1R in Osteocytes

Osteocytes constitute about 90% of all bone cells in adults [34]. These cells occupy a privileged site in the bone tissue, buried in the mineralized matrix and connecting among themselves and with other cells in the bone through dendritic processes in a complex lacunar–canalicular system [35]. Nowadays, osteocytes are considered the main orchestrator of bone remodeling, being able to control osteoblast and osteoclast function [35].

Several studies point to PTH1R as an important regulator in the control of bone anabolism by osteocytes [36,37]. Ostecytic osteolysis is the term used for resorption of bone matrix by osteocytes, and it has been associated with the rapid release of calcium from bone after an increase in PTH secretion for years [36].

Several studies knocking down or overexpressing PTH1R in osteocytes highlight its key role in these cells [32]. Deletion of PTH1R in osteocytes in a mouse model (DMP1-KOPTH1R) causes a low bone turnover phenotype with a moderate increase in bone mass, related to diminished RANK-L-dependent osteoclast activity [32,37]. Overexpression of a constitutively activated form of PTH1R in osteocytes (DMP1-CaPTH1R) induces a huge increase in bone mass and turnover [38]. In this model, inhibition of Wnt-signaling, by low-density lipoprotein receptor-related protein 5 (LRP5) deletion or

Box 2. Structural and Signaling Properties of PTH1R

PTH1R is a member of the class B1 GPCRs [69] that is expressed primarily in bone, kidney, and cartilage but also in blood vessels and several developing tissues and organs [70]. PTH1R activation induces different actions depending on the organ or cell type. In bone and kidney, PTH1R regulates serum calcium and phosphate levels and contributes to bone turnover and remodeling [71]. At the cellular level, PTH1R has shown to increase proliferation, survival, and differentiation of a variety of cells including osteoblasts, chondrocytes, osteocytes, and tumor cells [26,40,72,73].

PTH1R presents a large amino-terminal extracellular domain that is essential for ligand binding. By contrast, transmembrane domains and associated extracellular loop regions of PTH1R provide critical sites necessary for specific interactions with different PTH1R ligands. The intracellular loop IC3 contains the major regions required for specific G-protein coupling [74] and the carboxy-terminal intracellular tail presents different domains for direct interaction with scaffold and regulatory proteins, such as **β-arrestins** [25,75], sortin nexin 27 (SNX27) [76], NHERF [77], and dishevelled [78]. PTH1R triggers several intracellular signaling pathways initiated by the activation of different α subunits of heterotrimeric G proteins (Gαs, Gαq/11, Gα12/13, and Gαi [79]). (i) Gαs-dependent stimulation of adenylyl cyclase increases intracellular cAMP levels leading to increased PKA activity [80]. This, in turn, stimulates a variety of targets in bone cells such as c-fos and c-jun transcription factors [81]. (ii) Gαq/11-dependent stimulation of phospholipase (PL)C [82]. (iii) Gα12/13-dependent stimulation of RhoA and PLD [83]. (iv) Gαi-dependent cleavage of membrane-bound heparin-binding fragments of epidermal growth factor induces transactivation of the epidermal growth factor receptor and stimulation of mitogen-activated protein kinase (MAPK) ERK1/2 signaling pathway [84]. Alternatively, ERK 1/2 can be deactivated by PTH-dependent increase of MKP-1 phosphatase expression [85].

Activation of a particular signaling response pathways seems to depend on the expression of certain PTH1R-binding proteins including NHERF, disheveled, β-arrestin, and SNX27 proteins.

(i) NHERF proteins act as PTH1R scaffolds that affect PTH1R signaling by prioritizing the PLC pathway while inhibiting PKA activity through a Gαi-dependent pathway [77]. Moreover, NHERF-1 is absent in proliferating osteoblasts but is highly expressed in mineralizing osteoblasts, suggesting a key role in osteoblastic differentiation and matrix synthesis [86]. Thus, these effects could presumably be attributed to the NHERF-1-induced switch of PKA towards PLC signaling in differentiated osteoblasts.

(ii) Dishevelled interacts directly with PTH1R causing increased osteoblastic matrix mineralization and secretion of pro-osteoclastic factors by osteoblastic cells via activation of the β-catenin signaling pathway [78].

(iii) β-Arrestins play dual roles in PTH1R signaling regulation [87]. Phosphorylation of PTH1R by GPCR kinases (GRKs) follows agonist binding and induces β-arrestin coupling and subsequent desensitization of the receptor, terminating cAMP signaling upon stimulation with PTHrP. By contrast, β-arrestins participate in complexes that lead to prolonged cAMP responses in internalized vesicles with PTH stimulation [25]. Even though β-arrestins induce **receptor desensitization** of some PTH1R-dependent cAMP responses, recruitment of these proteins to PTH1R has been reported to form intracellular complexes that promote MAPK ERK 1/2 signaling [75].

(iv) SNX27, a protein involved in endosomal trafficking, has also been described to regulate PTH1R recycling following PTH stimulation. By promoting PTH1R association with endosomal sorting molecules (VPS retromer proteins), SNX27 facilitates rapid recycling of the receptor after stimulation with the agonist [76].

overexpression of sclerostin, the osteocyte-specific inhibitor for Wnt signaling, abolished the aforementioned high bone mass phenotype, but did not affect the increase in bone remodeling [38]. These studies suggest two different pathways downstream of PTH1R in osteocytes; one that leads to bone formation (sclerostin dependent), and one that leads to bone resorption (RANK-L dependent).

Moreover, the absence of PTH1R in osteocytes reduces the anabolic effects induced by intermittent administration of PTH in DMP1-KOPTH1R mice [32,37]. Another study points to the Gsα/cAMP as the specific pathway required for the increase in bone mass by PTH in the osteoblastic lineage [39]. Furthermore, PTH1R in osteocytes seems to be also needed for bone response to mechanical loading, as shown in DMP1-KOPTH1R where the anabolic actions of bone loading were impaired [37]. In this regard, we and others have recently showed that PTH1R acts as a mechanosensor and can be directly activated by mechanical stimulation [40]. In the osteocytic MLO-Y4 cells, a

ligand-independent PTH1R activation via an increased intracellular calcium influx, was found to occur immediately after mechanical stimulation by fluid flow [40]. Exogenous PTHrP(1–34) was shown to exert an additive anabolic effect with mechanical loading on diabetic mouse bone [41].

PTH1R in Chondrocytes

PTH1R activation by PTH inhibits osteoarthritis progression in mice [42] and rats [43], whereas PTHrP has been reported to maintain proliferation and inhibit the differentiation of **chondrocytes** during **endochondral ossification** [44]. Regarding **cartilage** regeneration, some reports suggest that activation of PTH1R negatively regulates the terminal differentiation of chondrocytes [43,45,46]. By contrast, PTH/PTHrP signaling during the early stages of the cartilage repair process induces regenerative chondrogenesis in articular cartilage defects [46]. Thus, whether a therapy based on PTH/PTHrP analogs could be used for cartilage repair requires further studies.

Use of Teriparatide and Abaloparatide

Teriparatide PTH(1–34) is distributed by Lilly as Forteo. Meanwhile, abaloparatide is produced by Radious Health whose trademark is Tymlos in the USA. This company has tried to obtain approval from the European Medicines Agency (EMA) without success for distribution of abaloparatide using the trademark Eladynos in the EU. Both drugs are administrated subcutaneously, 20 μg daily in the case of Forteo and 80 μg daily in the case of abaloparatide. Regarding treatment efficacy, preclinical studies carried out in rats and monkeys showed that abaloparatide was able to increase BMD as well as improve the bone microarchitecture and geometry [47,48]. Ovariectomized rats treated with abaloparatide showed increased femoral and trabecular bone volume, trabecular number and thickness, and decreased trabecular separation and connectivity density both in L4 vertebra and distal femur. Abaloparatide also produced a change in biomechanical properties, increasing maximum load, and the energy to cause failure in L4 vertebra and distal femur [47]. Likewise, treatment of ovariectomized cynomolgus monkeys (OVX-cynos) with abaloparatide produced similar effects and increased the serum levels of the bone formation marker procollagen type 1 N-terminal propeptide (PINP) or the biomarkers of bone turnover C- and N-terminal telopeptides. Abaloparatide treatment induced an increase of BMD in L1–L4, femur, and tibia, an increase in L3–L4 peak load and L5–L6 stiffness [48].

Clinical trials reported compelling evidence supporting the increasing of BMD with less resorption activity in patients treated with abaloparatide compared with PTH(1–34) [49–52]. Abaloparatide decreased the risk of new vertebral and nonvertebral fractures, increasing BMD [49]: in women aged >80 years, BMD increased 12.1%, 3.9%, and 3.6% in lumbar spine, hip, and femoral neck after 18 months of treatment, respectively [50]. However, in this group there was no significant risk reduction of nonvertebral and vertebral fractures. In another clinical trial, administration of 80 μg abaloparatide compared with 20 μg teriparatide in postmenopausal women produced an increase in BMD of 6.7%, 3.1%, and 2.6% vs 5.5%, 1.1%, and 0.5% in lumbar spine, femoral neck, and hip, respectively [51]. A clinical trial called Abaloparatide Comparator Trial in Vertebral Endpoints (ACTIVE) compared the efficacy of teriparatide and abaloparatide in patients with OP [49]. Both abaloparatide and PTH treatment reduced the incidence of new vertebral fractures in 86% and 80% of participants, respectively. Abaloparatide promoted a larger increase in BMD than PTH in both the femoral neck and total hip but not in the lumbar spine. Abaloparatide also showed a significant decrease in C-terminal telopeptide levels, a biomarker for bone turnover measurement (proportionally associated to osteoclastic activity), compared with teriparatide [49].

Health and Safety Concerns

Eladynos merchandising has been prohibited at this stage by the EMA in the EU (http://www.ema.europa.eu/ema/index.jsp?curl=pages/medicines/human/medicines/004157/smops/Negative/human_smop_001280.jsp&mid=WC0b01ac058001d127) based on the recommendation of the Committee for Medicinal Products for Human Use that determined that two recent studies supporting abaloparatide efficacy were not performed in full compliance with good clinical practice. This committee also argued that from a safety point of view Eladynos might produce palpitations

and increase heart rate, potentially representing a danger in postmenopausal women. Therefore, they concluded that the risks of its administration to postmenopausal women with OP outweighed the benefits.

However, as reported in the ACTIVE clinical trial, hypercalcemia was significantly lower with abaloparatide treatment than with teriparatide [49]. Furthermore, a study with biopsies of iliac crest in patients in the ACTIVE trial found no evidence of aberrant bone formation. Cortical porosity was also analyzed in these biopsies and it was found that abaloparatide and PTH showed similar higher levels of porosity compared to the placebo group [53]. One of the main concerns of anabolic therapies is the risk of increasing the incidence of tumors. Studies performed to test abaloparatide risk of increasing osteosarcoma in up to 2 years of treatment – the limit of teriparatide treatment to avoid tumorigenesis based on animal studies [54] – showed that the risk of osteosarcoma in abaloparatide treatment was similar to that with teriparatide [55]. Notably, increased risk of osteosarcoma was reported in animals treated with doses from four to 28 times greater than the doses used in regular treatment. In fact, it has been reported that incidence of osteosarcoma in patients treated with teriparatide is similar to the incidence in the general population [56].

Future Perspectives

Abaloparatide has recently been approved for the treatment of postmenopausal OP, but based on its osteoanabolic properties, it could potentially be used to target other skeletal disorders characterized by bone loss or damage (see Outstanding Questions).

Although OP is more common in women after menopause, excessive bone loss is also experienced by men over the age of 65 years, and by young women with loss of estrogens caused by excessive exercising and/or eating restrictions or by exposure to glucocorticoid medications, alcohol abuse, diabetes, or other factors that cause secondary OP [41,57]. Given that abaloparatide shows a prevailing anabolic versus resorptive profile, treatment with abaloparatide may improve bone mass and decrease the risk of bone fracture in the aforementioned less common osteoporotic bone diseases.

Another potential use of abaloparatide may be its enhancement of fracture healing. Promotion of endochondral and **intramembranous ossification** by activation of chondrocytes and osteoblasts are essential processes in fracture healing [58]. Previous reports have shown that treatment with PTH enhances both chondrogenesis and osteogenesis during bone repair in PTH-treated fractures [59]. Similarly, PTHrP has also been involved in osteogenesis during fracture healing [60] and is a well-known regulator of endochondral ossification [61], supporting the likely role of abaloparatide as a future regenerative factor in fracture healing.

OA is a cartilage degeneration disorder associated with aged individuals [62] that will likely have a growing impact on healthcare in the future due to the increased aging population. Previous findings have shown that PTHrP downregulation is involved in the pathogenesis of OA [63] and it has been suggested that abaloparatide could be a potential therapy in OA by inducing chondrogenesis via decreasing intracellular oxidative stress [64]. Furthermore, preclinical observations have described that teriparatide decelerates cartilage degeneration and induces cartilage matrix regeneration in patients with OA [65]. Based on the previously mentioned findings abaloparatide could be a potential candidate for OA treatment.

Other studies have focused on alternative uses of PTH/PTHrP analogs in dental indications. In either advanced periodontitis, that ultimately leads to periodontal bone loss, or in osteonecrosis of the jaw, in which antiresorptive medications used for OP or cancer weaken and cause bone degradation of the untreated jaw, the use of PTH1R analogs has been proposed [66].

Aside from alternative possible future targets, research has been conducted to improve efficacy of abaloparatide treatment in OP. In this regard, administration of abaloparatide followed by the bisphosphonate alendronate has been reported to enhance BMD and reduce fracture risk in

postmenopausal women [67], suggesting the beneficial actions of a therapy based on abaloparatide followed by antiresorptive drugs.

Another future challenge is to develop noninvasive routes of abaloparatide delivery to avoid painful hypodermic injections and improve patient compliance. Currently, a transdermal formulation of abaloparatide with administration via a microneedle patch is being developed to address this issue [68].

Concluding Remarks

There is a constant demand to incorporate new drugs that alleviate the effects of OP or that are able to increase bone mass. The beneficial effects of abaloparatide, namely inducing an increase of bone mass, lowering hypercalcemia without increasing side effects, and its lower cost compared with PTH, make this drug an attractive treatment for OP. The development of abaloparatide would have not been possible without discovering the selective activation of PTH1R, which highlights the importance of basic research for the development of new drugs. Given the characteristics of abaloparatide, future uses might be extended to treat nonmenopausal loss of bone diseases, fracture healing, OA, or dental diseases.

Outstanding Questions

Could abaloparatide be used as a therapy to prevent bone fractures by increasing bone mass in secondary and male OP?

Is enhanced healing of fractures a putative new use of abaloparatide?

Could abaloparatide treatment decelerate cartilage degeneration and induce cartilage matrix regeneration in OA?

Can bone degradation status in advanced periodontitis or in osteonecrosis of the jaw be ameliorated by abaloparatide treatment?

Could combined therapy with other drugs improve abaloparatide actions on OP?

Are there new noninvasive routes of administration of abaloparatide that could reduce pain while maintaining effectiveness? Could we develop alternative delivery systems to improve quality of life in patients with osteoporosis?

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