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Review

Targeting the osteoblast: approved and experimental anabolic agents for the treatment of osteoporosis

Konstantinos A. Toulis,[1] Athanasios D. Anastasilakis,[1] Stergios A. Polyzos,[2] Polyzois Makras[3]

[1]Department of Endocrinology & Metabolism, 424 General Military Hospital, Thessaloniki, [2]Second Medical Clinic, Medical School, Aristotle University of Thessaloniki, Ippokration Hospital, Thessaloniki, [3]Department of Endocrinology & Diabetes, 251 Hellenic Air Force & VA General Hospital, Athens, Greece

ABSTRACT

Targeting osteoblast may be the means of effectively improving both bone quality and mass, thus offering an intriguing alternative in the treatment of osteoporosis. Aside from injectable parathyroid hormone (PTH) and its novel preparations, PTH-related peptide (PTHrP), calcilytics, beta-adrenergic receptors, enhancement of Wnt signaling (mainly via sclerostin and Dickkopf-1 neutralization), regulation of low-density lipoprotein receptor-related protein (LPR) 5/osteoblast axis, activin, IGF-1, and bone morphogenic proteins (BMPs) are reviewed for their basic rationale and evidence of bone anabolic potential. Sclerostin neutralizing antibody, teriparatide transdermal patch, and PTHrP (1-36) are currently at an advanced stage of research. Safety and tissue specificity are the prerequisites in the development of a novel treatment, especially when addressing a chronic condition such as osteoporosis.

Key words: Anabolic treatment, Osteoblast, Osteoporosis, Parathyroid hormone, Sclerostin, Teriparatide

INTRODUCTION

In the normal skeleton, bone remodeling, the coordinated resorption and formation of skeletal tissue, is carried out by the osteoclasts and the osteoblasts, respectively, in the basic multicellular units. This process is necessary for the maintenance of calcium homeostasis, microdamage repair, adaptation to mechanical loading, and removal of the aged tissue.

Age-related bone loss, independent of sex steroid status, which occurs as early as the third decade,[1] is the net effect of an imbalance in bone remodeling such that bone resorption exceeds bone formation. This condition may eventually result in osteoporosis, which may be defined as the increased risk for fracture, resulting from decreased bone mass, along with deranged bone microarchitecture and compromised bone strength.[2] In fact, osteoporosis

Address for correspondence:
Dr. Polyzois Makras, MD, PhD, Department of Endocrinology & Diabetes, 251 Hellenic Air Force & VA General Hospital, 3 Kanellopoulou Str, 11525, Goudi, Athens,
Tel.: +30 210 7463606, Fax: +30 210 7715690,
e-mail: makras@internet.gr

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is the leading cause of fractures, and interventions addressing this increased risk are found to be cost-effective (http: //guidance.nice.org.uk/TA160). Since osteoporosis results from a bone remodeling state in which bone resorption exceeds bone formation, targeting osteoclasts (differentiation, proliferation, function, and life span) by antiresorptive agents, such as bisphosphonates, seems a reasonable choice. However, along with halting deterioration of bone microarchitecture, antiresorptive agents also inhibit formation, which is tightly coupled to resorption.[3] Thus, important bone quality properties cannot be restored by targeting osteoclast. Hence the question arises as to whether or not targeting osteoblast would be a more appropriate intervention.

Intermittent parathyroid hormone (PTH) administration, the only currently available agent specifically targeting osteoblast, not only attains documented efficacy in increasing bone mass and preventing fractures [4] but also improves bone quality.[5] It is plausible to assume that such benefits might be expected from an agent that targets osteoblastic lineage (osteoblast commitment, differentiation, proliferation, function, and/or life span). This review aims at providing an overview of the currently available anabolic therapies and an insight into promising investigational anabolic therapies for the treatment of osteoporosis.

PARATHYROID HORMONE

Background

Human PTH is an 84-amino acid peptide playing a central role in the maintenance of calcium homeostasis. Parathyroid cells sense extracellular calcium concentration via the calcium-sensing receptors (CaSR) and secrete PTH in response to calcium levels decrease. Subsequently, PTH mobilizes calcium from skeletal stores, stimulates release of calcium (and phosphate) by activation of bone resorption, increases renal tubular calcium reabsorption, and indirectly enhances intestinal calcium absorption via its stimulatory action on renal 1α-cholecalciferol hydroxylase.[6]

PTH is considered to have mixed catabolic and anabolic effects on the skeleton. Long-standing hyperparathyroidism causes osteoporosis of predominantly cortical bone (forearm and hip), while it relatively preserves cancellous bone (spine). On the other hand, intermittent subcutaneous (sc) administration of low-dose PTH results in a skeletal anabolic response, more obvious in the cancellous than in the cortical bone, due to direct effects on cells of the osteoblastic lineage, and indirect effects through the regulation of selected skeletal growth factors.[7] Although it appears that bone anabolic properties are fully maintained by the truncated fragment hPTH(1–31) or its cyclized lactam, the 34-amino acid peptide hPTH(1–34), teriparatide, is currently approved for the treatment of severe osteoporosis in the United States and Europe.[4] Full-length PTH (PTH 1-84) is approved for the same indication only in Europe. Currently, PTH is the only proven bone anabolic therapy, whereas data attributing bone anabolic properties to classic antiresorptive agents, such as bisphosphonates[8-10] or the strontium ranelate (originally considered as a mixed agent),[3] are not consistently replicated.[11] Intermittent PTH administration exerts its effects via several molecular actions,[7] including but not limited to prevention of osteoblast apoptosis,[12] induction of IGF-1 synthesis, inhibition of sclerostin expression,[13] activation of Wnt signaling,[14] and induction of transcriptional factors, such as runx2.[15] Intermittent PTH has been shown to increase (a) the osteoblast number and their activity, (b) the bone remodeling rate along with the amount of bone deposited in each remodeling cycle, (c) trabecular thickness and trabecular connectivity, and (d) cortical thickness and bone size.[6] Thus, PTH increases not only bone mass but also bone quality by improving microarchitecture and geometry.[5,16] This mode of action is different from that of antiresorptives, which mainly act by maintaining skeletal architecture and decelerating bone turnover.

Evidence for the usefulness of PTH as anabolic therapy in osteoporosis

Daily SC administration of 20 or 40 μg of teriparatide over a median duration of 19 months was associated with a statistically significant reduction in vertebral and non-vertebral fractures in women with low bone mass.[17] Since the higher dose scheme was associated with a similar effect on bone fragility but a significantly higher risk of adverse events (such as hypercalcemia and hypercalciuria), the 20 μg dose per day, associated with a 65% reduction in radiographic vertebral fractures, was selected for commercial use.

Daily SC administration of full-length PTH (1-84) over 18 months was associated with a statistically significant reduction in vertebral, but not in non-vertebral, fractures in women with postmenopausal osteoporosis.[18] Differences in efficacy observed between PTH analogs may be largely explained by differences in baseline characteristics between trials in regard to age and prevalence of vertebral fractures. Differences in adverse events, favoring teriparatide, were also evident; again, enrollment criteria might have played a role. Teriparatide could also be considered in the treatment of male osteoporosis[19] as well as in glucocorticoid-induced osteoporosis.[20]

Short-term side-effects of PTH treatment include dizziness, leg cramps, hypercalcemia and hypercalciuria. In the long term, a putative risk for osteosarcoma has been reported in rat models with long-term (near lifetime) administration of doses many times higher than the doses approved for humans.[21,22] On the basis of this observation, and in accordance with recent studies,[23] treatment duration with PTH analogs should not exceed 24 months, although small series have recently been published with treatment up to 36 months.[24] Their use should not be considered in patients with Paget's disease, prior skeletal irradiation, unexplained elevations of bone-specific alkaline phosphatase, and adolescents with open epiphyses. To date, there is no evidence of increased risk of osteosarcoma in humans with either of the two available PTH preparations. Another debatable issue is the effect of PTH treatment on the cortical bone; either no change or a decrease in bone mineral density (BMD) at cortical bone sites (radius) has been reported with PTH treatment.[17,25] These changes may be attributed to a decrease in secondary mineralization of newly formed osteoid and are probably counteracted by an increase in cortical bone diameter (since the strength of a cylinder is proportional to the fourth power of its radius). This "paradox" may be indicative of the suboptimal estimation of bone strength and integrity only on the basis of areal bone mass measurements, age, and prevalence of vertebral fractures.

Comments

Although new bone formation with PTH therapy could be regarded as the "holy grail" in osteoporosis treatment, certain parameters, associated with PTH therapy, may attenuate the optimism. First, an "anabolic window" is observed with PTH treatment, namely increases in bone-formation markers are followed by analogous increases in bone-resorption markers,[26] and thus coupling occurs and a new balance is established. Second, PTH is a last resort therapy[6] since its maximal use does not exceed 24 months in a lifetime, whereas treatment of osteoporosis far exceeds this time frame. Third, it is an expensive treatment and is recommended as an alternative treatment option for the secondary prevention of fractures only in postmenopausal women who are (a) 65 years or older and have a T-score ≤-4.0 SD, or a T-score ≤-3.5 SD plus more than two fractures, or who are aged 55–64 years and have a T-score ≤-4 SD plus more than two fractures and (b) intolerant of oral bisphosphonates and strontium ranelate (http://guidance.nice.org.uk/TA161/Guidance). Furthermore, hard evidence on hip fracture reduction is still lacking for both preparations. Moreover, adherence and persistence to daily injections seem to decline after the first 6-month period,[27] a parameter which may undermine treatment efficacy. There is also a need for resolution-of-effect data, namely evidence on what happens when treatment is discontinued.[28] Finally, patients may be reluctant to initiate a therapy that requires daily injections.

Novel PTH preparations

In an attempt to overcome the compliance issues, alternative methods of PTH administration (oral, transdermal, nasal) have been tested. PTH (1-34) was formulated with the absorption enhancer 5-CNAC to provide an oral PTH preparation, named PTH134. In a Phase I, single-center, partially blinded, incomplete cross-over trial (NCT00676312) reported in 2009 at the ACR/ARHP Annual Scientific Meeting (DOI: 10.1002/art.25967), PTH134, at doses of 2.5 or 5 mg, provided systemic exposure levels approximating those of teriparatide 20 μg SC showed a comparable incidence of adverse events in 32 healthy postmenopausal women. Another study of similar design in osteoporotic/osteopenic postmenopausal women, using bone markers as the main outcome measure, is currently recruiting (NCT01224717). Research regarding transdermal PTH delivery is at a more advanced stage. PTH transdermal patch is composed of a small adhesive patch coated with PTH (1–34)

(TPTD-P).[29] In a 6-month, randomized, placebo-controlled trial (RCT) in 165 postmenopausal women, TPTD-P (20, 30 or 40 μg dose) significantly increased total hip BMD compared to both placebo patch and teriparatide injection and lumbar spine (LS) BMD *vs* placebo patch in a dose-dependent manner.[30] A nasal spray formula of PTH (1-34) provided encouraging results in a 3-month, uncontrolled, open-label pilot study in 90 osteoporotic subjects using LSBMD as the main outcome measure.[31] However, the subsequent, Phase II, 6-month, active-controlled trial was terminated prior to enrollment for unknown reasons (NCT00624481).

Aside from alternative routes of PTH administration, different PTH analogs have been developed in an attempt to achieve a safer and more potent profile. ZT-031 (ostabolin-C) is a cyclic 31-amino acid PTH analog which when administered by daily SC injections to postmenopausal women with osteoporosis, resulted in a dose-dependent increase in LS and total hip (TH) BMD without significant adverse events.[32] For all the above preparations, anti-fracture efficacy, safety, and tolerability should be documented before this treatment may be considered as alternative to standard therapy.

PTH-RELATED PEPTIDE (PTHrP)

Background

PTHrP acts as a paracrine regulator in several tissues, including cartilage, mammary, developing tooth, central nervous system, and smooth muscle.[33] It is also considered the most common cause of humoral hypercalcemia of malignancy.[34] Although PTHrP and PTH are products of different genes, with sequence divergence at the amino acid and nucleotide level, limited overall sequence homology (16%), and fundamentally differing physiology, they can activate a common G-protein coupled receptor, the PTH/PTHrP receptor (PTH1R) in their target cells, such as osteoblasts and renal tubular cells.[35] This is explained by the significant homology in their N-terminal, where nine out of their 13 amino acid residues are identical. Given the common receptor in the skeleton, it could be postulated that truncated PTHrP could exert actions similar to those of PTH.

Evidence for potential usefulness of PTHrP as anabolic therapy in osteoporosis

In preclinical models, PTHrP -/- mice died postnatally, probably from asphyxia, and exhibited widespread abnormalities of endochondral bone development.[36] PTHrP+/- mice by age 3 months presented with osteopenia characterized by altered trabecular architecture.[37] In PTHrP+/- mice, daily administration of the 1-34 amino-terminal fragment of parathyroid hormone (PTH 1-34) resulted in profound improvement in all parameters of skeletal microarchitecture,[38] establishing a role of PTHrP in bone formation in a PTH-like manner. In mice and rabbit models, the important role of PTHrP was documented in both endochondral and intramembranous bone formation.[39] What's more, synthetic human PTHrP stimulated bone resorption and caused hypercalcemia in rats.[40]

In humans, daily SC PTHrP (1-36) produced reduction in serum phosphorus and the renal phosphorus reabsorption threshold, increments in fractional calcium excretion and nephrogenous cAMP excretion, and increases in plasma 1,25-dihydroxyvitamin D.[41] Daily SC PTHrP (1-36) administration for 14 days in 13 postmenopausal women provided evidence of uncoupling bone formation from resorption, as assessed by relevant markers.[42] In a 3-month double-blind RCT, 16 postmenopausal women with osteoporosis were administered daily SC PTHrP (6.56 mcg/kg/d, or approximately 400 μg daily), along with calcium and vitamin D. The PTHrP group displayed a 4.7% increase in LS BMD, an increase in serum osteocalcin, and no change in markers of osteoclastic bone resorption.[43] In a dose escalation study in healthy volunteers, SC PTHrP (1-36) was found safe in single doses up to 2.0 mg.[44] In a subsequent 3-week dose escalation clinical trial in 41 healthy postmenopausal women, PTHrP administration in the dose of 500 and 625 μg daily was not associated with serious adverse events, whereas those on 750 μg developed mild hypercalcemia, attributed to activation of intestinal calcium absorption by 1,25 dihydroxyvitamin D and not to activation of bone resorption.[45] A 3-month, comparison trial of PTH (1-34) and two different doses of PTHrP (1-36) is currently recruiting postmenopausal women with osteoporosis to assess the stimulation of bone formation in relation to bone resorption (NCT00853723).

Comments

PTH is considered as a mixed skeletal anabolic and catabolic agent and its use has been limited by nausea, muscle cramping, and hypercalcemia.[45] The latter is associated with increases in osteoclast-driven bone resorption. Thus, an agent that preferably stimulates bone formation over bone resorption might circumvent these problems.[45] However, although PTHrP did not result in increased bone resorption markers in human studies, it is quite possible that this effect was caused by the limited period of administration, which could probably correspond to the "anabolic window" of PTH treatment. On the other hand, evidence regarding PTHrP anti-fracture efficacy is still lacking and SC administration does not address the major issue of convenience of treatment in order to maximize compliance.

CALCILYTICS

Background

The secretion of PTH by the parathyroid glands is tightly regulated by a G protein-coupled receptor (GPCR), the calcium-sensing receptor (CaSR). In humans, loss-of-function CaSR mutations are associated with autosomal dominant familial hypocalciuric hypercalcemia (FHH), whereas gain-of-function mutations are associated with autosomal dominant hypocalcemia (ADH).[46]

CaSR is highly expressed in the chief cells of the parathyroid gland where it is involved in the regulation of PTH gene-expression, PTH secretion, and parathyroid gland hyperplasia.[47] CaSR activation by extracellular Ca^{2+} leads to inhibition of PTH secretion.[48] Antagonism of the CaSR would mimic a state of hypocalcemia and elicit a PTH pulse as a compensatory mechanism.[49] Given that intermittent rather than continuous PTH exposure promotes anabolic rather than catabolic effect on the skeleton, it has been suggested that transient, short-acting antagonists of the CaSR, resulting in transient, rapid bursts in PTH, would favor new bone formation, mimicking intermittent PTH administration. The negative allosteric modulators of the CaSR, which right-shift the concentration-response curve of Ca^{2+}, are known as calcilytics. Of note, an orthosteric antagonist of the CaSR has not yet been identified.[50]

Evidence for potential usefulness of calcilytics as anabolic therapy in osteoporosis

In preclinical models, several oral compounds with CaSR antagonizing properties, including NPS 2143, Calhex 231, and SB-423557, have been tested for their efficacy to stimulate a PTH "spike" and increase bone formation in ovariectomized rats.[48,49,51,52] A structure similar to NPS 2143, ronacaleret hydrochloride, demonstrated a convenient pharmacokinetic profile and reached Phase II to assess its safety and efficacy.[53] In this 12-month, double-blind, placebo and active-controlled (alendronate and teriparatide) trial, efficacy of ronacaleret hydrochloride (100, 200, 300, 400 mg daily) was assessed using surrogate markers of anti-fracture efficacy (LS and TH BMD, bone turnover markers, bone-strength parameters). However, that trial and further development were terminated due to lack of efficacy, which was attributed to a variety of factors such as poor exposure, limited ability of dose escalation due to safety concerns or lack of potency.[54] Recently, a follow-up observational study (CR9112792) after ronacaleret discontinuation was completed. This study used the percent change in LS BMD measured by dual x-ray absorptiometry (DXA) as the primary outcome and the results are to be published.

Most recently, two novel structural classes of oral calcilytics were reported. Both of them showed a favorable pharmacokinetic profile of PTH stimulation in osteopenic ovariectomized rats[55,56] and dogs.[56]

Comments

The most appealing characteristics of the calcilytics are (1) the oral administration, which circumvents the major problem of consistency and adherence to daily injections of PTH, and (2) the stimulation of the natural PTH, as opposed to teriparatide or synthetic PTH 1-84. However, it should be noted that CaSR is expressed in other tissues, such as kidney, bone cells,[57] heart.[58] In fact, it has been suggested that CaSR might be one of the mediators of strontium ranelate effects on bone cells.[59] At doses used for the antagonism of the parathyroid cell CaSR leading to "a dramatic increase in bone turnover", calcilytics do not interact with the bone cell CaSR.[49] However, this might not be the case for the heart cell CaSR, which is worrisome, especially in the light of new evidence

suggesting that CaSR antagonism in the heart may impair cardioprotective ischemic preconditioning.[58]

BETA-BLOCKERS

Background

Bone is innervated by, autonomous nervous system and osteoblasts express the beta-2 adrenergic receptors (beta2AR).[60] Evidence from mice models suggests a central regulation of bone mass by the sympathetic nervous system (SNS), mediated through leptin-regulated neural pathways.[61,62] The exact mechanisms involved in this type of regulation of bone mass, involving norepinephrine, acetylcholine, leptin, neuropeptide Y, neuromedin U, endocannabinoids, and serotonin interactions, have not been fully elucidated as yet. In general, SNS activation is considered to contribute to bone loss.

Beta-Adrenergic receptor antagonists (beta-blockers - BB) are established antihypertensive agents and their actions result from a reduction in cardiac output and in renin activity and inhibition of the catecholamines peripheral action on beta-adrenergic receptors.[63] It appears that BB may also have a role in bone metabolism.

Evidence for potential usefulness of BB as anabolic therapy in osteoporosis

In preclinical models, deletion of one or both copies of the beta2AR increased bone mass in mice.[61] Treatment with the low-dose agonist isoprenaline, a nonspecific beta-AR agonist, induced bone loss mainly via enhanced bone resorption in mice,[64] whereas low-dose propanolol, a non-selective BB, increased bone formation in a rat model.[65]

In humans, the potential role of BB in osteoporosis has largely been based on retrospective database analysis. A large, registry-based, case-control study from the UK, which included 30,601 case subjects (defined as such with any incident fracture) and 120,819 appropriately matched controls, provided evidence that use of BB is associated with a reduced risk of fractures, either alone (OR: 0.77, CI 0.72-0.83) or in combination with thiazide diuretics.[66] Using the same UK database, with the addition of the Dutch database and a different definition of cases (a first hip or femur fracture), researchers concluded that the use of BB was associated with a reduced risk of hip/femur fracture in both (UK and Dutch) study populations (adjusted OR = 0.82, 95% CI 0.74-0.91, and adjusted OR = 0.87, 95% CI 0.80-0.95, respectively). Moreover, the investigators concluded that this risk reduction was not associated with the cumulative dose or BB selectivity but with the past use of other antihypertensive agents.[67] Subsequently, using the same Dutch database, but investigating the effect of beta-2 agonists instead of BB, researchers found an association between higher doses of beta-2 agonists and significant risk of hip/femur fracture, although this excess risk was substantially reduced after exclusion of oral glucocorticoid users and adjustment for the underlying disease.[68] Accordingly, a relevant meta-analysis of seven observational studies associated the use of BB with a significant reduction of any type of fracture [Relative Risk 0.86, 95% Confidence Interval (CI) 0.70-0.98].[69] In a case-control study using as a reference population 944 postmenopausal women referred for BMD measurement, BB users had higher BMD and a borderline significantly lower risk for fractures at all sites (odds ratio 0.56; 95% CI: 0.30-0.99).[70] In a prospective, population-based study of 1,793 subjects with mean follow-up of around 11 years, the association between the use of BB and incidence of any fracture was significant after adjustment for a variety of potential effect modifiers [Hazard ratio 0.60; 95% CI = 0.37-0.96).[71] In a recent prospective cohort study of 3,488 individuals, the same association (use of BB associated with lower fracture risk) was confirmed and was found to be robust in selective over non-selective BB users and independent of sex, age, BMD, and clinical risk factors.[72]

Comments

The evidence on the effects of BB on the human skeleton is largely derived from observational studies which cannot establish a cause-effect relationship and hence further validation is required. Assumptions on the exact mechanism of their effect may be projected from preclinical models, favoring an anticatabolic over an anabolic action. In humans, evidence suggests that BB use is associated with significantly higher LS and FN BMD, higher cortical width, and higher mean H parameter, an index that reflects a better trabecular microarchitecture.[70] Moreover, an elegant experiment in humans demonstrated a PTH burst as a response

to selective beta-1 adrenergic blockade by esmolol,[73] mimicking the effects of CaSR antagonism. Despite these data, the role of BB in osteoporosis has not as yet been adequately elucidated.

ENHANCEMENT OF WNT/B-CATENIN SIGNALING PATHWAY

Background

Wnts (Wingless tail) or Wnt ligands constitute a family of secreted, lipid-modified, cysteine-rich glycoproteins which play an important role in the regulation of cell differentiation and proliferation.[74] In osteoblasts, Wnt ligands can act either through the Wnt/β-catenin canonical pathway or the non-canonical (β-catenin independent) pathways. The latter include Wnt/planar cell polarity signaling, the Wnt-cGMP/Ca^{2+} pathway, and a protein kinase A pathway but the net effect of their activation on bone metabolism remains to be elucidated.[75] On the other hand, activation of the β-catenin dependent canonical pathway is considered to (i) promote osteoblast commitment from the multipotential, mesenchymal progenitors, (ii) stimulate osteoblast proliferation and differentiation at the expense of osteoclastogenesis, and (iii) prevent both osteoblast and osteocyte apoptosis.[76] These actions render the Wnt/β-catenin canonical pathway and its modulators a promising target for treating low-bone mass disorders.[77]

In brief, cytoplasmic β-catenin levels are normally kept low through continuous degradation.[78] In the absence of Wnt ligands, β-catenin is phosphorylated by kinases, forming the "β-catenin destruction complex", and is subsequently degradated. Glycogen synthase kinase-3β (GSK3β) plays a major role in this complex along with the protein axin, the adenomatous polyposis coli (APC) protein, and the casein kinase 1. Phosphorylated β-catenin is then ubiquitinated before final proteosomal degradation.[79] In the presence of Wnt ligands (Wnt1, Wnt3A, Wnt8, Wnt10b), β-catenin degradation is inhibited, resulting in its accumulation in the cytoplasm and its translocation into the nucleus, where nuclear β-catenin interacts with T cell-specific transcription factor/lymphoid enhancer-binding factor 1 (TCF/LEF) to promote the transcriptional response of Wnt target genes.[15] The cascade of events that leads to β-catenin stabilization in the cytoplasm is a receptor-mediated response in which Wnt ligands interact with a member of the Frizzled family (Fzd) and the co-receptor lipoprotein-receptor related protein (LRP) 5 or 6.[80,81] The formation of a receptor complex (Wnt-Fzd and LRP5/6) results in inhibition of β-catenin phosphorylation by GSK-3β through an intracellular process that involves the cytoplasmic protein dishevelled (Dsh/Dvl) and axin.[78]

The Wnt/β-catenin canonical pathway is modulated by a complex network of extracellular antagonists, transmembrane modulators or intracellular signals.[82] In fact, it has been suggested that the "non-canonical" Wnts (Wnt4, Wnt5a, and Wnt11) may antagonize Wnt/β-catenin signaling.[83] In humans, known antagonists of the Wnt/β-catenin canonical pathway include Wnt Inhibitory Factor 1 (WIF-1), secreted frizzled related proteins (sFRPs), Dickkopf (Dkk) proteins, and sclerostin. WIF-1 and sFRPs are extracellular proteins which bind directly to Wnt proteins, thus preventing them from activating the Wnt/β-catenin canonical pathway.[84] sFRPs act as decoy receptors by mimicking Fzd structure. On the other hand, Dkks and sclerostin target LRP5/6 mediation of Wnt signaling. Dkks (Dkk1 and Dkk4, but not Dkk2) by binding to transmembrane Dkk receptors (Kremens proteins) and form a complex that attracts and internalizes LRP5/6, disrupting the Wnt/β-catenin canonical pathway.[85,86] Sclerostin, the product of the SOST gene, produced almost exclusively by osteocytes, binds to LRP5/6 domains and antagonizes LRP5/6-mediated Wnt signaling.[87] The Wnt/β-catenin canonical pathway, in its inactive and active form, is depicted in Figure 1.

Certain genetic disorders provide interesting insights into the effects of the enhancement or disruption of the Wnt/β-catenin pathway in *in vivo* models. The osteoporosis-pseudoglioma syndrome (OPPG) is a rare autosomal recessive disorder of severe juvenile osteoporosis and congenital blindness due to loss-of-function mutations in the LRP5 gene (disruption of Wnt/β-catenin pathway).[88,89] A single amino-acid substitution (G171V) in the same gene, which prevents Dkk1 binding, leads to high bone mass disorders.[90,91] Six other amino-acid substitutions in the aminoterminal part of LRP5 protein also resulted in high bone mass phenotype.[92] Similarly, a missense mutation in LRP6 leads to autosomal dominant early cardiovascular disease (CAD), hyperlipidemia, hyperten-

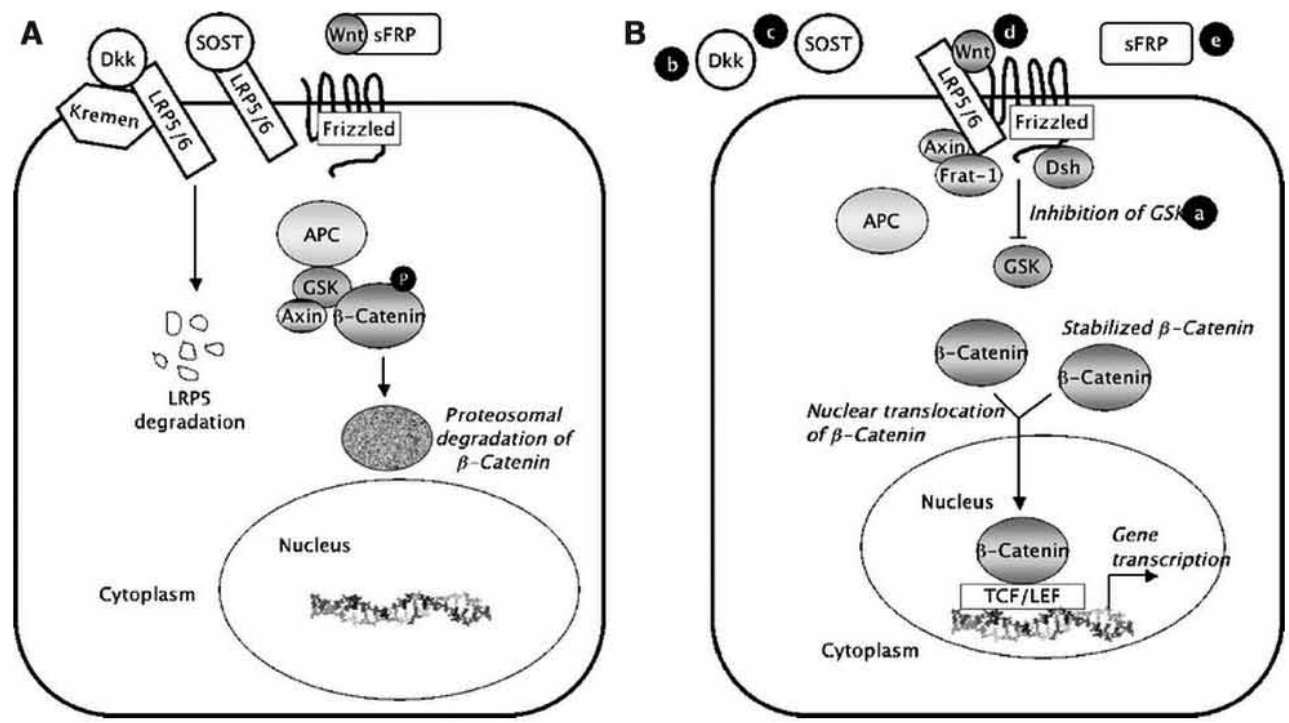

Figure 1. Overview of the Wnt signaling pathway in the inactive and active state. (A) Wnt signaling pathway in the inactive state, showing a ligand Wnt inhibited by a decoy secreted frizzled-related protein (sFRP), the co-receptor, low-density lipoprotein receptor-related protein (LRP) 5/6, bound by either inhibitory protein, sclerostin or Dkk (Dickkopf), and Glycogen synthase kinase (GSK)-3β in the active state, resulting in proteosomal degradation of β-catenin. (B) Active Wnt signaling, with LRP5/6 engaging in receptor complex after Wnt binding, disruption of GSK-3β inhibitory complex, stabilisation of β-catenin, and its translocation to nucleus where it activates transcription. Reproduced with kind permission from Springer Science + Business Media: Osteoporosis International, regulatory pathways revealing new approaches to the development of anabolic drugs for osteoporosis, 19: 2008, pp. 1125–38, Martin TJ, Sims NA, Ng KW, Figure 2.

sion, diabetes, which and osteoporosis (disruption of Wnt/β-catenin pathway).[93] Sclerosteosis and van Buchem disease, which are rare sclerosing bone dysplasias caused by inactivating mutations in the SOST gene[94,95] and deletion in regulatory elements of SOST transcription,[96] respectively, result in absence of sclerostin expression and progressive generalized osteosclerosis. Patients with sclerosteosis have a more severe phenotype compared to patients with van Buchem disease and usually have syndactyly,[97] while the gene carriers have increased BMD.

Evidence for potential usefulness of Wnt/β-catenin pathway components as anabolic therapy in osteoporosis

Several components of the Wnt/β-catenin pathway could be potential targets for osteoanabolic treatment (Figure 1B). Those at the most advanced stage of development, namely sclerostin neutralization, Dkk1 neutralization, and GSK-3β inhibition, will be reviewed herein.

In *in vivo* models investigating sclerostin neutralization, 5-week treatment with a sclerostin neutralizing monoclonal antibody (Scl-AbII) increased bone formation at all sites in aged ovariectomized rats,[98] aged male rats,[99] and primates (cynomolgus monkeys).[100] Two sclerostin monoclonal antibodies (Mabs) (AMG 785 and AMG167) have been developed and are currently under investigation in humans. In a 3-month, phase I, double-blind RCT, 72 healthy men and postmenopausal women were subjected to a single injection of either AMG 785 or placebo in a 3:1 ratio.[101] AMG 785 was administered SC (n=42) or intravenously (n=12) in sequentially increasing dosages and was generally well tolerated. A dose-dependent increase in LS and TH BMD and

in bone formation markers (P1NP, osteocalcin, bone specific ALP) was observed. The largest increase in LS (5.3%) and TH (2.8%) BMD at 3 months was observed with the highest SC dose of 10 mg/kg. In the same cohort, over two-fold increase in bone formation markers at 3-4 weeks and a sustained reduction of approximately 50% in serum C-terminal type 1 collagen telopeptide (sCTX) were observed. Of note, no subjects received calcium or Vit D supplementation, which may have resulted in underestimation of AMG 785 efficacy.[101] The magnitude and pattern of changes in bone markers implies a rapid uncoupling of bone formation - resorption and possibly a prolonged "anabolic window".[102] A Phase I trial of similar design is currently ongoing in a healthy Japanese population (NCT01101061). On the basis of these findings and the pharmacokinetic/pharmacodynamic (PKPD) profile assessed in the Phase I trial, SC administration at 4 weeks intervals and doses of 70, 140, and 210 mg have been selected for further investigation in a Phase II trial. A 12-month RCT (NCT00896532) was designed, using a sample size of 419 postmenopausal women with low BMD, percent change from baseline at month 12 in LS BMD as the primary outcome and placebo, alendronate, and teriparatide as comparators. Furthermore, a sclerostin antibody (Scl-AbIII) increased bone formation during metaphyseal repair, but also in untraumatized bone in rats.[103] Therefore, AMG 785 is currently under investigation for its efficacy in functional healing in patients with intertrochanteric fractures of the proximal femur (NCT01081678), in radiographic fracture healing in patients with tibial diaphyseal fractures (NCT 00907296), and for its effect on the bone quality of the forearm in postmenopausal women with low bone mass (NCT00950950). As for AMG 167, a Phase I, ascending single-dose, double-blind RCT to evaluate its safety, tolerability, and PKPD in healthy men and postmenopausal women has been completed (NCT00902356). This study is being followed by another Phase I, multi-dose trial in men and postmenopausal women with low BMD and no history of fragility fracture, which is currently ongoing (NCT01101048) and will include calcium and Vit D supplementation.

Four to six week treatment with an anti-Dkk1 antibody increased BMD and the number of osteoblasts in a mouse model of multiple myeloma.[104] In humans, an investigational anti-Dkk1 antibody (PF-04840082) has been developed. In elegant preclinical PKPD profiling experiments, intravenous PF-04840082 lowered Dkk-1 levels in a dose-dependent manner and it was estimated that 0.008 mg/kg/day, could be the starting dose with the minimal biological effect in humans.[105] A 3-month Phase I study to evaluate the safety, tolerability, immunogenicity, and PKPD of escalating doses of IV RN564 in women with osteopenia and in healthy men has been announced (NCT01293487); percentage changes from baseline in LS, TH, FN, and distal radius BMD at Day 85 are included as a secondary outcome.

In *in vivo* models investigating GSK-3β neutralization, 4 weeks of treatment with the GSK-3β inhibitor, lithium chloride, resulted in increased bone formation and bone mass in Lrp5(-/-) mice.[106] Treatment with an oral dual GSK-3a and -3b inhibitor (LY603281 - 31 – 8) for 2 months resulted in increased markers of bone formation, bone mass, and strength in ovariectomized rats.[107] Given the involvement of GSK-3 in significant signaling pathways, the probable lack of tissue specificity in GSK-3β inhibition and side-effects associated with lithium, the development of a GSK-3β inhibitor for osteoporosis treatment in humans seems difficult at present.[14,15]

An alternative means of Wnt/β-catenin pathway activation, involving antagonism of sFRP-1, has recently been reported in *in vitro* experiments in which diarylsulfone sulfonamide (WAY-316606), by binding to sFRP-1, increased total bone area in murine cultures.[108]

Comments

Safety and tissue specificity are the prerequisites and key goals in the development of any treatment, especially when addressing a chronic condition such as osteoporosis. Indirect evidence from clinical observation and basic data suggest that both of them (safety and tissue specificity) may be anticipated in sclerostin neutralization. In fact, patients with sclerosteosis and van Buchem disease, where sclerostin is absent, live a fairly normal life, with the exception of skeletal manifestations and those secondary to nerve compression (safety).[97] Furthermore, sclerostin is produced almost exclusively by osteocytes (tissue specificity). Thus, it comes as no surprise that the

pharmaceutical neutralization of sclerostin is actively sought for and currently at the most advanced stage of development among all candidate molecules targeting Wnt/β-catenin pathway.

PERIPHERALLY DERIVED 5-HYDROXYTRYPTAMIN (HT)

Background

Serotonin or 5-HT is derived from tryptophan and is found either in the periphery (gastrointestinal tract, platelets) or in the central nervous system, exhibiting distinct functions, depending on the site. Peripheral 5-HT is synthesized in enterochromaffin cells of the gut, with an isoform of tryptophan hydroxylase (Tph1) playing the role of rate limiting enzyme, and then stored in platelets. Brainstem-derived 5-HT (BDS) is synthesized locally under the control of another isoform of Tph (Tph2); the bloodbrain barrier does not allow 5-HT to cross in either direction, thus allowing them to act in relative isolation.[109] 5-HT has recently attracted great interest in bone biology after experimental data suggesting that it could be part of a novel endocrine axis regulating bone mass, and consequent therapeutic implications.[110] Experiments using conditional gene deletion and microarray in mice demonstrated that LRP5, the alleged co-receptor in the Wnt/β-catenin pathway, may promote bone anabolism in a Wnt-independent manner by inhibiting the expression of Tph1 in the duodenum.[111] Furthermore, BDS favors bone mass accrual after its binding to medial hypothalamic neurons and this bone anabolic action is inhibited by leptin, providing a link between central regulation of bone mass, energy expenditure, and appetite.[112] However, recently published experiments by another laboratory failed to replicate these findings.[113]

Evidence for potential usefulness of 5-HT as anabolic therapy in osteoporosis

In preclinical models, a decrease in 5-HT blood levels with an inhibitor of 5-HT synthesis (parachlorophenylalanine) or a low-tryptophan diet resulted in normalization of bone formation and bone mass in Lrp5-deficient mice. Gut- but not osteoblast-specific Lrp5 inactivation resulted in a decrease in bone formation. What's more, inactivation of Tph1 or gut-specific activation of Lrp5 prevented ovariectomy-induced bone loss and increased bone mass.[114] Similarly, an oral inhibitor of both Tph-1 and Tph-2, LP533401, which decreases peripheral 5-HT levels, when administered to ovariectomized rodents for up to 6 weeks increased bone formation only and the subsequent increase in bone mass was dose-dependent.[115] In addition, LP533401 was as effective as PTH in preventing the bone microarchitectural changes after ovariectomy.[115] However, another laboratory reported no significant difference either in bone mass between Tph1-/- and wild type mice or in bone mass of ovariectomized mice after treatment with LP923941(Tph-1 inhibitor).[113] Finally, fluoxetine (a selective serotonin reuptake inhibitor – SSRI), when administered to animal models, negatively affected bone accrual by reducing bone formation without increasing bone resorption;[116] no significant effects in bone quality were observed after 6-month exposure.[117]

In humans, indirect evidence of the effect of 5-HT on the skeleton was sought in cross-sectional, case-control and prospective cohort studies, using BMD and fractures as outcome measures. However, different methodology applied for the measurement of 5-HT, the direct effect of medication on the sense of balance (a risk factor for falls), and other confounding factors suggest caution in the interpretation of the results. Although in cross-sectional analysis of NHANES III (7,114 male and 7,532 female participants) and of Women's Health Initiative Observational Study (82,410 women) antidepressants (including SSRI) were not associated with a significantly reduced BMD,[118,119] several other studies reported the opposite in either gender.[120-122] Several studies of variable design reported a significantly increased dose-specific risk of fractures associated with the use of SSRIs,[119,121,123-128] with discordant results in regard to skeleton site. Finally, a recent population-based observational study suggested that in women, serum (but not platelet-poor plasma) 5-HT levels were weakly inversely correlated with indices of bone strength, as assessed by DXA, quantitative computed tomography (QCT), and high-resolution peripheral QCT (pQCT).[129]

Comments

Recent evidence suggested a dual nature of 5-HT activity depending on the site of synthesis (central vs. peripheral); according to this, peripheral 5-HT has

a negative effect on bone mass, whereas BDS has a positive effect. These findings were not replicated in recent experiments[113] and hence more data are needed before considering peripherally derived 5-HT as a potential target for the development of novel anabolic agents.

ACTIVIN ANTAGONIZING AGENTS

Background

Activins are produced by pituitary cells and gonads and stimulate pituitary FSH release, while inhibins prevent activins from binding to their receptor type IIA (ActRIIA) in the gonadothophs, thereby suppressing FSH release; follistatin, another regulator of the system, binds and neutralizes activin. Activin, inhibin, and follistatin are members of the TGF-b superfamily.[130] Age-related reduction in inhibin B and follistatin levels, subsequent increase in FSH, and sustained levels of activin A have been implicated in the pathogenesis of fast bone loss in perimenopausal women.[131, 132]

Activin A is expressed in bone, is abundant in the extracellular bone matrix, and seems to regulate both osteoblastogenesis and osteoclastogenesis.[133] Its role in osteoblastogenesis is somewhat controversial. Activin A enhances the induction of ectopic bone formation when implanted concurrently with bone morphogenetic proteins (BMPs),[134] increases osteoblast proliferation and collagen synthesis,[135] increases the thickness of periosteum and bone matrix layers when injected into the periosteum,[136] and stimulates fracture healing[137] in animal models. By contrast, several studies demonstrated an inhibitory effect of activin A on osteoblast differentiation in murine, rat, and human cell cultures *in vitro*.[138-140]

On the other hand, activin A seems to exert a stimulatory effect in osteoclastogenesis and increases bone resorption via ActRIIA signaling;[141-143] this could also result indirectly through stimulation of FSH release, which appears to promote osteoclastogenesis as well.[144,145] In contrast, inhibins inhibit osteoclastogenic differentiation in bone marrow cultures,[141] while they increase osteoblast differentiation in mice bearing the human inhibin A gene.[133] Therefore, pharmacological blockage of activin signaling has been proposed as a target for the treatment of osteoporosis.

Evidence for potential usefulness of activin antagonizing agents as anabolic therapy in osteoporosis

Activin antagonists represent molecules that bind to activin and prevent it from binding to its endogenous receptor.

In preclinical models, a fusion protein of the extracellular domain of the ActRIIA linked to the Fc portion of murine IgG2a (RAP-011) stimulated bone formation, resulting in increased bone mass and strength in intact and ovariectomized mice.[146] A similar soluble chimeric form of activin receptor type IIB (ActRIIB) fused to a murine IgG2aFc subunit was also tested and prevented loss of bone mass in gonadectomized male mice as assessed by whole body DXA and micro-computed tomography of proximal tibias.[143] Another fusion protein of the extracellular domain of the ActRIIA linked to human IgG1-Fc (sotatercept – ACE-011) has also been developed. Within only 3 months, biweekly subcutaneous ACE-011 at a dose of 10mg/kg in primates increased bone mass by 13-15% and trabecular bone volume over 70%.[146] In another study in primates, ACE-011 increased bone formation rate and osteoblast surface and decreased osteoclast surface and number at all sites (vertebrae, femoral neck, and distal femur), indicating a dual model of action on the skeleton.[147]

In healthy postmenopausal women, a single-dose of ACE-011 (0.01-3.0 mg/kg IV and 0.03-0.1 mg/kg s.c.) dose-dependently decreased FSH levels, increased serum bone-specific alkaline phosphatase (bone formation), and decreased levels of CTx and tartrate-resistant acid phosphatase (TRACP)-5b (bone resorption).[148] ACE-011 was well tolerated.

Comments

To date, the efficacy of all available osteoporosis treatments (both anabolics and antiresorptives) is limited by the coupling effect. The dissociation between bone formation and resorption in favor of the former would lead to rapid and more significant bone mass increases. Activin antagonist ACE-011 is an agent with proven uncoupling effect in humans in that it increases bone formation while decreasing bone resorption. This may eventually mark a new class of agents in osteoporosis treatment, provided that this effect is verified in larger studies.

BONE MORPHOGENETIC PROTEINS

Osteoblasts have receptors for BMPs which are, like activin A, members of transforming growth factor-β superfamily. BMP synthesis is not limited to bone and BMPs are expressed by a variety of extraskeletal tissues, where they play a critical role in organ development and cell function. BMPs were originally identified because of their ability to induce endochondral bone formation.[149] BMPs induce the differentiation of mesenchymal stem cells (MSCs) toward cells of the osteoblastic lineage, increasing the pool of mature osteoblasts and enhancing their differentiation. Furthermore, BMPs induce endochondral ossification and chondrogenesis.[150] BMPs also induce the transcription of osteoprotegerin, a decoy receptor that limits the effect of receptor activator of nuclear factor-κB-ligand (RANKL) on osteoclastogenesis.[149] BMP-2, BMP-4, and BMP-6 are the most readily detectable BMPs in osteoblast cultures. However, there are exceptions: BMP-1 is a metalloprotease whose action is unrelated to other BMPs.[150] In contrast, BMP-3 or osteogenin inhibits osteoblastogenesis.[151]

BMPs act through a cell membrane receptor complex. BMP binding to either the ActR2 dimer or BMP receptor type 2 (BMPR2) induces co-association of Activin receptor type 1 (ActR1) dimer. This results in a conformational change that enables Smad-1, Smad-5, and Smad-8 proteins to bind Smad-4, and the whole Smad-complex to enter the nucleus to drive gene transcription.[152] Apart from Smad activation, BMP receptor complex may also regulate gene transcription through mitogen-activated protein kinase (MAPK) activation. Extracellular antagonists, including noggin, gremlin, and twisted gastrulation, bind BMPs or components of the BMP signaling pathways and prevent their signal transduction. The antagonists are regulated by BMPs, indicating the existence and need for local feedback mechanisms to temper BMP cellular activities, given that unopposed BMP effects may be detrimental.[149] It has also been proposed that BMP signaling induces sclerostin expression, thereby sclerostin is included in feedback mechanisms to self-limit BMP-induced excessive bone formation.[153]

Evidence for potential usefulness of BMPs as anabolic therapy in osteoporosis

BMPs play an important role in the advancement of bone engineering strategies. They have been used in the management of acute fracture, delayed fracture healing, arthrodesis, spinal fusion, and nonunion. Specifically, recombinant human (rh)BMP-2 and rhBMP-7 have been approved in several countries for specific indications, previously reviewed.[154,155] The theoretical basis of local or systemic treatment of osteoporotic fractures with BMPs includes the rapid increase in bone strength locally at the fractured area or their action on the entire skeleton, when given systemically, as well as their acceleration of the bone-healing period.[156]

In preclinical models of osteoporosis, BMP-2, BMP-4, BMP-6, and BMP-7 have mainly been studied. rhBMP-2 induced bone formation in rat models, but its effect declined with age.[157] In a mouse model,[158] rhBMP-2 induced bone formation; the observed bone mass increase was associated with an increase in MSCs numbers, osteogenic activity and proliferation, and a decrease in apoptosis. Similarly, systemically administered ^{125}I-BMP-6 increased bone volume and mechanical characteristics of both the trabecular and cortical bone, thereby improving microarchitecture and quality of the skeleton in osteoporotic rats.[159] Locally applied rhBMP-7 treatment improved mechanical strength and histomorphometric parameters of osteopenic vertebra in an ovine model; however, these changes were not consistently associated with changes in BMD.[160] Finally, *ex-vivo* gene therapy to deliver BMP-4 improved bone healing in critically sized fractures of osteoporotic rats.[161]

Apart from direct BMP administration, BMP indirect upregulation may represent promising targets. Osthole, a coumarin-like derivative extracted from Chinese herbs, has been shown to stimulate osteoblast proliferation and differentiation by both activating Wnt/β-catenin signaling and increasing BMP-2 expression in a rat model of osteoporosis.[162] Ursolic acid, derived from ubiquitous plant triterpenoid, was shown to have bone-forming activity in a mouse model, possibly through increasing BMP-2.[163]

Piceatannol, a polyphenol present in grapes and wine, increased BMP-2 synthesis, thereby inducing osteoblasts differentiation and increasing bone mass.[164] Furthermore, naringin, a polymethoxylated flavonoid, was reported to increase BMP-2 expression and enhance osteogenesis.[165]

Other anabolic targets might be the inhibition of BMP antagonists' action. Silencing of noggin expression enhances new bone formation induced by rhBMP-2 in a mouse model, thereby proving useful for intensifying the effects of BMPs in promoting new bone formation.[166] Similarly, deletion of gremlin gene resulted in sensitization of BMP signaling and activity and in enhancement of bone formation in mice.[167]

Comments

Despite favorable preclinical data and clinical data from orthopedic surgery, there are no clinical studies reporting the effect of recombinant BMPs administration or inhibitors of BMP antagonists in patients with osteoporosis. One Phase II clinical trial, comparing the effect of locally administered recombinant BMP-2 plus bisphosphonate versus bisphosphonate alone on BMD in postmenopausal women with low bone mass and high risk for hip fracture, is currently ongoing (ClinicalTrials.gov Identifier: NCT00752557).

Adverse events of BMP administration are reported in orthopedic studies. The reported data are limited and include mainly heterotopic ossification, but also minor immunogenic reactions, swelling, and infections.[168] However, given that BMPs are critical for the differentiation and function of many cellular systems besides the skeleton, rhBMP administration or inactivation of BMP antagonists might be lethal or result in severe developmental abnormalities.[149]

It is expected that BMPs will be more intensively investigated in the preclinical and clinical setting of osteoporosis in the near future. Apart from cost and safety considerations, a technical problem that should be solved before integrating BMPs into the active osteoporosis research is the need for systemic administration. The majority of clinical orthopedic studies are performed with locally acting injections of BMPs on the site of fracture or fusion or nonunion and, therefore, are not directly applicable to osteoporosis, since osteoporosis is a systemic generalized rather than local metabolic disease. Therefore, the discovery of specific BMP-carriers would be of major importance for BMP-focused clinical trials. Silk fibroin microparticles have been used as carriers of rhBMP-2 in a rat model[169] or poly-L-glycolic acid biospheres for rhBMP-7 in an ovine model[160] with considerable success.

GROWTH HORMONE (GH) AND INSULIN GROWTH FACTOR-1 (IGF-1)

Background

The GH-IGF-1 axis exerts a predominantly anabolic effect on the skeleton, with the increase in bone formation surpassing the increase in bone resorption.[170] The declining activity of the GH-IGF-1 axis with advancing age may contribute to the decrease in bone mass that occurs with aging.[171]

Evidence for potential usefulness of GH or IGF-1 as anabolic therapy in osteoporosis

In vitro studies demonstrated that both GH and IGF-1 increase bone collagen,[172] while IGF-1 additionally stimulates the synthesis of bone DNA and non-collagen proteins.[173] Furthermore, both molecules stimulate bone growth and osteoblast activity in animals.[174,175] In some[176-178] but not all[179] studies, BMD of the LS was somewhat lower in untreated patients with adult-onset GH deficiency compared with normal subjects. The degree of osteopenia appears to correlate directly to the degree of GH deficiency. However, the number and severity of other hormonal deficiencies are also more pronounced in patients with more severe GH deficiency,[180] making it difficult to determine which factors are the most important in the development of osteopenia. In elderly women but not in men higher serum IGF-1 levels were associated with higher BMD.[181]

Administration of GH to GH-deficient men and women has resulted in improvement in BMD in some[171,182] but not all studies.[183,184] Most of these studies did not have a placebo control group and virtually all used GH doses that resulted in supraphysiologic serum GH concentrations. In three randomized trials, GH treatment increased spine BMD in men but not in women.[182,185,186] In non-GH deficient men and women with normal BMD,[170,187] osteopenia[188] or osteoporo-

sis,[189-191] GH administration has yielded conflicting results, whereas side-effects, such as hyperglycemia, hypertension, arthralgia, and the carpal tunnel syndrome, were common.[171,187-191] These results plus the inconvenience of administration by daily injection rendered GH treatment unsuitable for patients with osteoporosis who are not GH-deficient. On the other hand, IGF-1 therapy appeared to be ineffective.[192]

Comments

Osteoblasts have IGF-1 receptors and direct or indirect (through GH administration) delivery of IGF-1 in these cells could result in an anabolic effect on the skeleton. However, although locally acting IGF-1 seems to play a decisive role in bone formation and many of the above mentioned therapies, including PTH, may exert their action, at least in part, through induction of IGF-1 synthesis,[193] there is no convincing evidence to date that SC GH or IGF-1 administration may constitute a promising osteoanabolic agent.

CONCLUSION

Targeting osteoblast could offer novel approaches for the improvement of bone strength and reversal of age-associated bone loss not currently addressed by the classic antiresorptive therapies. Since the approval of PTH use in the treatment of osteoporosis, recent advances in basic bone biology, including but not limited to the understanding of the Wnt/β-catenin pathway, LRP5/osteoblast pathway, CaSR, PTHrP, and BMPs have revealed novel potential targets that could lead to the discovery of novel anabolic agents. An overview of these targets in the osteoblast and the osteoblast progenitor cell is illustrated in Figure 2. Among them, sclerostin neutralizing antibody, teriparatide transdermal patch, and PTHrP (1-36) seem to be at the most advanced stage of research (Table 1). Since osteoporosis is a chronic condition, safety and tissue specificity are prerequisites in the development of a novel treatment, especially when it affects molecular signaling pathways.

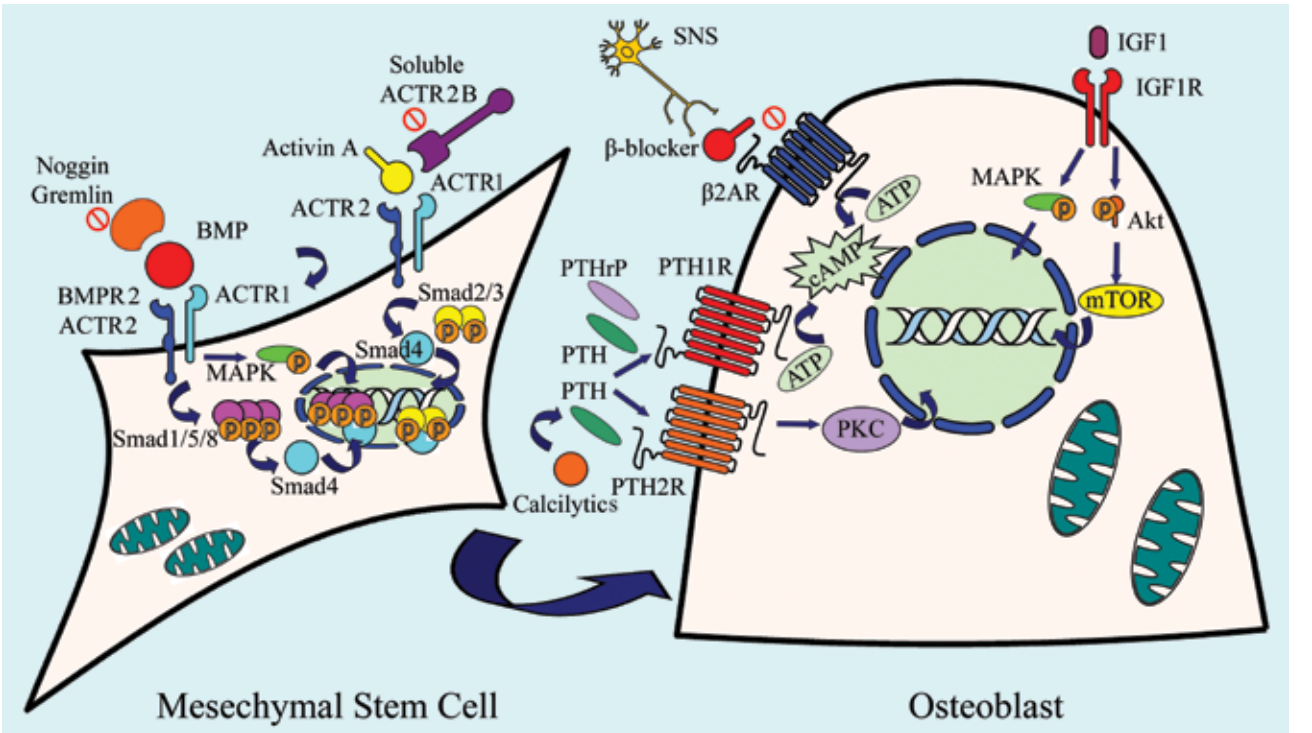

Figure 2. Molecular targets of the current and experimental bone anabolic therapies in the osteoblast and/or its progenitor cell. ACT: activin-type; ATP: adenosine triphosphate; β2AR: b2-andrenergic receptor; BMP: bone morphogenic protein; cAMP: cyclic adenosine monophosphate; IGF1: insulin-like growth factor 1; mTOR: mammalian targer of rapamycin; MAPK: mitogen-activated protein kinase; R: receptor; PKC: protein kinase C; PTH: parathyroid hormone; PTHrP: PTH-related peptide; SNS: sympathetic nervous system.

Table 1. Current and selected investigational bone anabolic therapies in clinical trials

Name	Active molecule	Mode of administration	Dose(s)	Indication	References and/or ClinicalTrials.gov identifier	Manufacturer/Sponsor
Phase III trials and beyond						
PTH (1-34) (teriparatide)	1-34 amino acid PTH analog	SC inj	20 μg daily	PO, MO, CIO	(16), (18), (19)	Eli Lilly and Company
PTH (1-84)*	Full-length PTH analog	SC inj	100 μg daily	PO	(17)	NPS Pharmaceuticals, Inc
Phase II trials						
TPTD-P	Adhesive patch coated with teriparatide on a titanium microneedle array	Transdermal patch	20, 30 or 40 μg in 30 minutes wear-time	PO	(28)	Zosano Pharma Inc
PTHrP(1-36)	PTH related peptide analog	SC inj	400 μg or 600 μg daily	PO	(43), (NCT00853723)	University of Pittsburgh
AMG 785	Sclerostin neutralizing monoclonal antibody	SC inj	70, 140 and 210 mg	PO	(99) (NCT00896532)	Amgen Inc
rhBMP-2/CPM	Bone morphogenic protein -2 in injectable calcium phosphate matrix	intraosseous inj	1.0 and 2.0 mg / mL as supplementation to bisphosphonates	PO	(NCT00752557)	Wyeth
Phase I trials						
ZT-031 (ostabolin-C)	Cyclic 31-amino acid PTH analog	SC inj **	7.5, 15, 30 or 45 μg	PO	(30)	Zelos Therapeutics and Aegis Therapeutics
AMG 167	Sclerostin neutralizing monoclonal antibody	SC inj	70, 140 or 210 mg daily (women) and 70 or 210 mg (men)	PO, MO	(NCT01101048)	Amgen Inc
RN564	Anti-DKK1 monoclonal antibody	IV infusion	Escalating doses	PO	(NCT01293487)	Pfizer Inc
PTH134	Teriparatide formulation with absorption enhancer 5-CNAC	Per os	2.5 or 5 mg	PO	(NCT01224717)	Novartis Pharmaceuticals
Trials terminated at Phase II						
SB-751689 (ronacaleret hydrochloride)	CaSR negative allosteric modulator (antagonizing)	Per os	100, 200, 300 or 400 mg	PO	(NCT00471237) [A follow-up observational study in (CR9112792)]	GlaxoSmithKline Inc and NPS Pharmaceuticals, Inc
PTH (1-34) nasal spray	Teriparatide formulation	Intranasally (spray)	Dose-Ranging	PO	NCT00624481	Nastech Pharmaceutical Company, Inc.

PTH: Parathyroid hormone; SC: subcutaneous; inj: injectable; PO: postmenopausal osteoporosis; MO: Male osteoporosis; CIO: corticoid-induced osteoporosis; PTHrP: Parathyroid hormone related peptide; Inc: Incorporation; IV: Intravenous; Dkk1: Dickkopf protein 1; mg: milligram; μg: microgram; CaSR: Calcium Sensing Receptor.
*Approved in Europe; **Manufacturer (Zelos) announced an intranasal administration system to be investigated for both ostabolin-C (ZT-034) and teriparatide (ZT-031)

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