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Roles of growth factors, calcitonic polypeptides and neuropeptides in bone metabolism, osteoporosis and rheumatis arthritis

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【Abstract】 Osteoporosis is a common disorder characterized by reduced bone mineral density, deterioration of the microarchitecture of bone tissue and increased risk of fracture. The aim of treatment of osteoporosis is to maintain and, ideally, to restore bone strength safely. In recent years the role of polypeptide growth factors in bone metabolism has begun to appear. It has been proposed that alterations in the expression or production of growth factor can modulate the proliferation and activity of bone forming cells. Thus, the role of structurally diverse peptides for the management and diagnosis of osteoporosis has attracted the attention of many investigators. This paper reviews numerous findings concerning the use of polypeptides, hormones, and growth factors, for the management of osteoporosis. Many of the compounds mentioned here are experimental prototypes of new therapeutic classes. Though it is unlikely that some of the compounds may ever be used clinically, development of safe and efficacious agents in each class will define the future course of therapy for osteoporosis.

Key words : Bone mineral density; Bone remodeling and coupling; Growth factors; Calcitonins; Polypeptides; Neuropeptides

Introduction

Osteoporosis is a metabolic disorder characterized by low bone mass and microarchitechtural deterioration of bone structure, resulting in bone fragility and increased susceptibility to fracture [1]. By current definitions, osteoporosis is defined as bone mineral density (BMD) > 2.5 standard deviations (SD) below the young adult mean value [2]. Osteoporosis fractures occur most commonly in the hip, spine, distal radius and ribs. The incidence of these fractures is higher in women than in men and increases sharply after 50 years of age [3]. Low bone mass is considered to be the main characteristic of these fractures. Many risk factors for osteoporosis have been identified in cross-sectional studies. These include family history, hormonal factors, inadequate nutrition, intake of medication, immobility and

diseases [2]. Prospective epidemiologic studies indicate that BMD is still the single best predictor of fractures [4] for women at the time of menopause, possibly with the addition of a few risk factors. For elderly persons, the combination of risk factors for falling and low BMD is probably more predictive than a BMD measurement alone. Though the exact cause of bone loss in patients with osteoporosis is not yet clear, it is considered to be mediated primarily by a single cell type called an osteoclast. These bone reabsorbing osteoclasts are of hemopoietic origin, probably of the monocyte macrophage family. Theoretically, increased activity of osteoclasts or decreased activity of bone forming osteoblasts or both may be associated with osteoporosis. Recent studies have shown that genetic factors may have a major role in the pathogenesis of osteoporosis and segregation analysis has shown that BMD is under polygenic control [5,6,7]. The genes encoding collagen types I α1 and I α2 (COLIA 1 and COLIA 2) are also important candidates for the genetic regulation of bone density and population based studies have shown that COLIA 1 polymorphism is associated with reduced bone density and predisposes wom

Osteoporosis is among the most wide-spread afflictions of industrialized countries and costs approximately $13.8 billion each year to the US health care system alone [8]. It affects more than 75 million people in Europe, the US and Japan [9] and it is estimated that approximately 40 in 100 women will experience one or more fractures after the age of 50 [10]. These fractures are associated with high morbidity and disability. Statistics show that the occurrence of osteoporosis is on the rise with continued growth of the elderly population and may result in the doubling or tripling of the number of fractures in next century [11]. Of all fragility fractures, the number of hip fractures world-wide are projected to increase almost fourfold from 1990 to 2050 [10], resulting in an increased burden on the US health care system, in addition to causing pain, disability, and a reduced quality of life.

Current osteoporosis treatments are not curative and historically have relied on estrogens (hormone replacement therapy, HRT) as the mainstay of drug treatment, but the risks of breast cancer versus the cardiovascular and skeletal benefits remains the major cause of concern. The bisphosphonates and, possibly, fluorides are likely to be the major alternatives to estrogens. Recent studies have shown that dietary supplementation with calcium and vitamin D may be beneficial in men or women 65 years of age or older. However, for many patients treatment with HRT, calcium and vitamin D have been contraindicated or ineffective [12]. Use of a combination treatment, e.g., estrogen and bisphosphonates together, is still under clinical evaluation. Many new agents for the treatment of osteoporosis are currently being examined. First line therapies include aldendronate (bisphosphonate) and calcitonin. Presumably these agents act directly through effects on the skeletal system. However, there is a possibility that these effects may be more complex in nature than a single increase in BMD and that effects on remodeling space, as well as on nonskeletal factors, may also be important in the mechanism of their influence on fracture risk. Though the ideal drug is yet not available, growth factors, that affect bone metabolism and are derived from

the bone, hold great promise in the near future for the development of clinically acceptable therapeutic agent.

The purpose of this review is to outline the very recent advances, attributed to an entirely novel class of polypeptide-based therapeutic approaches for the treatment of osteoporosis. This review has been structured in sections, each dealing with either peptides, peptidyl hormones or polypeptide growth factors that can pharmacologically intervene in the molecular events of osteoporosis. Many of the compounds mentioned here are experimental prototypes of new therapeutic classes. Though it is unlikely that some of the compounds may ever be used clinically, development of safe and efficacious agents in each class will define the future course of therapy for osteoporosis. Many nonpeptidyl compounds such as ERT, bisphosphonates, calcium, and fluorides being used or developed for the treatment of osteoporosis have been recently reviewed by several authors and will not be discussed here [13].

I. Classification of osteoporosis, and Bone remodeling and coupling

Clinically, osteoporosis can be classified in several ways and its management varies in accordance with the classification. Postmenopausal osteoporosis is experienced by a large percentage of elderly women, possibly due to estrogen deficiency, and is referred to as type I osteoporosis [14]. Type II osteoporosis, also known as senile osteoporosis, occurs mostly in individuals over 70 years of age or in women 20 years after menopause. It is generally characterized by a gradual shrinking of skeletal mass that is often slow and age related [14]. Another form of osteoporosis, juvenile osteoporosis, occurs mostly in children between 8 and 14 years of age. It causes bone pain, fractures and is often associated with hypercalciurea.

Bone is a complex and dynamic tissue that undergoes continuous remodeling throughout life and the rate of remodeling increases in older adults. Three types of cells that are involved in the bone remodeling are: 1) Bone-lining cells or osteoprogenitor cells: resting cells about to be used to produce bone, located on internal bone surfaces. 2) Osteoblasts: bone-forming cells present on the surface of the developing bones. 3) Osteoclasts: large multi-nucleated bone reabsorbing cells formed by the fusion of osteoprogenitor cells. Although osteoclasts and osteoblasts are derived from different stem cells, their activity is highly coupled.

Bone loss occurs due to a negative bone balance at the level of the basic multicellular unit (BMU), i.e. the amount of bone reabsorbed exceeds the amount replaced, thereby resulting in too little bone or osteoporosis. Thus the rate of bone loss can be determined by the size of the imbalance at each BMU and also by the rate that new remodeling sites are created [15]. Bone remodeling occurs at discrete sites within the skeleton and proceeds in an orderly fashion, with bone reabsorption always being followed by bone formation, a phenomenon referred to as coupling. This remodeling depends on local factors, such as cytokines and growth factors that play an important role in the bone tissue as mediators of cell-to-cell and matrix-to-cell communication. Growth factors released from the bone matrix during the reabsorption are responsible for the refilling of the reabsorption

cavity by osteoblasts.

When an antireabsorptive agent is given, bone turnover is reduced. BMD then increases because bone formation initiated in the preceding remodeling cycle fills the remodeling space created during that cycle. In general, the increase in BMD is about 4-7% in the first 1-2 years. The higher the bone turnover, the greater the increase in BMD [15]. The initial increase in BMD is then followed by bone loss at a rate determined by the effect of the drug on bone turnover and on bone reabsorption and formation at BMU. If the BMU imbalance is abolished, bone loss will cease, irrespective of the rate of bone turnover. If a drug makes bone balance positive at the BMU by increasing the amount of bone formed so that it exceeds that is reabsorbed, BMD will continue to increase. Histomorphometry is needed to establish whether reabsorption depth is reduced and bone formation is increased. Thus an antireabsorptive drug should increase BMD by filling the remodeling space, reducing the rate of bone turnover, and reducing the imbalance at the level of the BMU.

II. Pathogenesis of osteophorosis and rheumatis arthritis

There could be many factors [16] that may influence osteoporosis. It could be a predisposed diseased condition, drugs, environmental influences, hormonal imbalance, alcoholism, smoking, dietary conditions or genetic factors. Many systemic and local factors, discussed in the following section, have also been identified [17,18] that affect bone metabolism. A knowledge of these factors is beneficial in understanding the mechanism of the disease, its treatment and the development of new agents for osteoporosis.

1) Role of hormones in bone metabolism

Impaired absorption of calcium, resulting in a negative balance, has been implicated with age-related bone loss. In the absence of adequate level of calcium, parathyroid hormone (PTH) is released that promotes bone reabsorption. Serum concentrations of PTH increase with age [19,20]. However, this increase is not entirely related to bone loss or to an increased incidence of vertebral fractures. Whereas high concentration of PTH inhibits bone formation, in low doses it can increase trabecular bone mass [21].

1,25-Dihydroxy vitamin D3 has been found to augment intestinal absorption of calcium in older patients with a lowered serum level of 1,25-dihydroxy vitamin D3 [22].

Calcitonin is an inhibitor of bone reabsorption. However, its excess or deficiency in patients does not cause a change in bone mass [23]. Also its level is not lowered in patients suffering from osteoporosis [24]. Decreased levels of calcitonin may be associated with those of testosterone and could be involved in osteoporosis in men [25].

Glucocorticosteroids exhibit complex effects on bone metabolism[26]. Under in vitro conditions, at lower concentrations, glucocorticosteroids increase osteoblastic collagen synthesis, whereas at high concentrations, bone formation is decreased, possibly due to inhibition of replication and differentiation of osteoblast precursors [27]. In in vivo situations it causes a decrease in bone formation that may cause osteoporosis. Deficiency of estrogens in

women and androgens in men [23,25] in the development of osteoporosis is well established. Withdrawal of these hormones causes increased bone reabsorption and a lesser increase in bone formation [28]. Progestins may also be involved in bone formation [16]. Stimulation of osteoblast formation [29] and inhibition of PTH could be reasons for the observed effect in reducing bone reabsorption. Prolactin also has been reported to have a positive effect in bone formation.

2) Role of growth factors in bone metabolism

Cartilage and bone tissues are rich in different polypeptide factors that participate in the regulation of skeletal development and growth. Parallels between the embryonal and endochondral ossification, callus formation during fracture repair, and ectopic bone induction in postnatal life have encouraged scientists to search for common mechanisms underlying the processes. Various growth factors have been identified in human bone that, in one way or other, have been found to play a major role in the repair of segmental bone defects and in fracture repair. In fact, these growth factors stimulate the proliferation and activity of bone cells and can stimulate bone formation. Data from various laboratories suggest that bone growth factors may act to couple bone formation to reabsorption to maintain bone mass during remodeling. Among these factors, insulin-like growth factors (IGFs), transforming growth factors (TGFs) and bone morphogenic proteins (BMPs) are important regulators of bone metabolism and will be described later. Other growth factors such as fibroblast growth factors [30], platelet growth factors and macrophage stimulating factor [31], that have been found to play an important role in the coupling of bone reabsorption and formation, are being investigated. It has been observed that alterations in the expression or production of growth factors can modulate the proliferation and activity of bone forming cells. Clinical efficacy of some of the growth factors has been studied that suggests that low doses of growth factor may stimulate bone formation. Thus, preventive or curative treatment of osteoporosis with growth factors appears to have a great therapeutic potential. Other local factors involved are cytokines and lymphokines [32], interleukin-1 (IL-1) [33], interferon-34, and others that directly or indirectly influence bone metabolism.

Ⅲ. Factors in bone metabolism

1) b-alanyl-histidinato zinc (AHZ)

The pathophysiological role of zinc in unloading-inducing osteopenia has been well documented [34]. Exogenous administration of zinc has been found to have an activating effect on bone formation and calcification both in vitro [35] as well as in vivo [36]. Recently a novel zinc-chelated dipeptide for delivering zinc has been reported to be more effective than zinc sulfate on bone metabolism [37]. In in vivo experiments with this compound, ovariectomized rats exhibited complete prevention of bone loss after treatment by an oral route (10-100 mg/kg). In vitro studies with AHZ also showed complete inhibition of the decrease of bone calcium in a bone tissue culture system, as well as in the formation of osteoclast-like cells in mouse marrow culture. It has been proposed that AHZ may stimulate

bone protein synthesis through the mechanism that is involved in protein kinases [38].

2) N-(benzyloxycarbonyl)-phenylalanyl-tyrosinal

Another low molecular weight pharmacophore, that exhibited significant suppressive effect on bone reabsorption both in vitro and in vivo, was identified in an aldehyde derivative of a dipeptide. In the assay for cysteine protease inhibitor, this synthetic dipeptide was found to be a potent and selective cathepsin L inhibitor. In another in vitro assay with unfractionated rat bone cells, 1.5 nM of the dipeptide derivative exhibited marked inhibition in parathyroid hormone-stimulated osteoclastic bone reabsorption. In addition, intraperitoneal (IP) administration of the peptidylaldehyde (2.5-10 mg/kg) in ovariectomized rats for 4 weeks prevented bone weight loss in a dose dependent manner. Hydroxyproline measurement of the decalcified femurs from these treated animals showed that this compound acts as a bone reabsorption suppressor through the inhibition of collagen degradation [39].

3) RGD peptides as blockers of integrin receptor

Integrins, a class of cell surface adhesion glycoproteins, play a key role in the attachment of bone reabsorbing cell osteoclasts to the bone mineralized matrix [40]. Most integrins bind to their ligands via the RGD tripeptide present within the ligand sequence. $\alpha v \beta 3$ vibronectin-like receptors present on the osteoclast cell surface play a major role in the attachment of osteoclasts to the reabsorption surfaces. Thus, development of an antagonist based on the RGD structure for all surface adhesion molecules, particularly the $\alpha v \beta 3$ vibronectin like receptor, may provide new treatments for osteoporosis. Extensive studies with linear and cyclic peptides containing the RGD sequence on bone formation and reabsorption have been carried out in different laboratories. They were found to inhibit osteoclasts (45Ca release) in 3 different in vitro reabsorption assays [41]. Recently a novel peptide mimetic, SC56631, based upon the $\alpha v \beta 3$ ligand, RGD, was found to recognize the isolated integrin, and its relative, $\alpha v \beta 5$, as effectively as the natural peptide. The peptide mimetic was found to reduce osteoclastic bone reabsorption both in vitro and in vivo. Most importantly, IV administration of the mimetic prevented the 55% loss of trabecular bone sustained by rats within 6 week of oophorectomy. Histologic examination of bones taken from SC56631-treated, oophorectomized animals also supported the compound's bone sparing properties and its capacity to decrease osteoclast number [42]. Thus, an RGD mimetic is able to prevent the rapid bone loss that accompanies estrogen withdrawal.

4) Echistatin

Echistatin, a 49-amino acid RGD-containing polypeptide, was originally isolated from the venom of the viper E. carinatus. It was found to interact with the osteoclast $\alpha v \beta 3$ integrin receptor and was able to block osteoclast attachment to bone and inhibit bone reabsorption in vitro. In vivo [43] studies also showed that echistatin could completely inhibit osteoclast mediated bone reabsorption. Structure-activity relationship studies are being carried out [44] with a view to identifying shorter and more potent antagonists of the $\alpha v \beta 3$ integrin that may find clinical application in the blockade of

bone reabsorption.

5) Cathepsin K inhibitors

Cathepsin K, a cysteine protease papain of the superfamily unique to osteoclasts, has been implicated in the process of bone reabsorption [45]. Selective inhibitors of cathepsin K therefore could be promising therapeutic agents for the treatment of osteoporosis. Recently Veber et al. [46] have designed 2 novel classes of potent and selective active site spanning inhibitors for cathepsin K. They act by mechanisms that involve tight binding intermediates, potentially on a hydrolytic pathway. The unique binding mode of the inhibitors has been detailed by X-ray crystallography and may be a close approximation of tetrahedral intermediates that occur during substrate proteolysis. The inhibitors were found to exhibit antireabsorptive activity in vivo and in vitro and, therefore, are promising leads as therapeutic agents for the treatment of osteoporosis.

6) Growth hormone (GH)

The role of GH in promoting longitudinal bone growth prior to epiphyseal closure is well established. However, its use for the treatment of postmenopausal osteoporosis (PMO) is still debated. A number of clinical studies have shown that administration of GH leads to the activation of osteoclasts and osteoblasts along with an increase in bone mass as evidenced by an increase in biochemical markers of bone reabsorption or formation [47]. However, the mechanism by which this is achieved is not known. Recent extensive studies pertaining to its therapeutic efficacy, as well as mode of action, have been carried out in various laboratories. Kassem et al. have studied the responsiveness of bone cells to exogenous hormonal stimuli in humans with PMO and observed no major disturbance in osteoclastic and osteoblastic responsiveness [48].

Recently, 2 groups have independently reported their observations with respect to the therapeutic efficacy of GH, by administering recombinant hGH in PMO women. Combined treatment with calcitonin over a period of 24 months was able to maintain bone mass at lumber spine and distal radius, but induced a decline at femoral shaft [49]. Similarly, another group administered GH in an acyclic manner with or without calcitonin and found a statistically significant increase in bone mineral density of the lumber spine and hip; however, they were less pronounced than those achieved with estrogen or bisphosphonates [50]. Wright et al. [51] studied the effects of GH on serum 1,25 dihydroxyvitamin [$1,25(OH)_2$ D] and observed a modest increase in serum $1,25(OH)_2$ D that is mediated by IGF-I and is independent of parathyroid hormone.

Thus, at present, GH does not appear to be a useful tool for the treatment of PMO due to its poor overall effect on bone mineral density. However, it may be of interest to study the efficacy of growth hormone releasing hexapeptide (GHRP-6) and its potent congeners [52,53,54] that are known to release GH in a circadian, pulsatile fashion in humans and are undergoing clinical trials as growth promoters.

7) Calcitonins

Calcitonins (CTs) are polypeptide hormones that are secreted by the parafollicular C-cells of the thyroid that prevent skeletal breakdown by inhibiting the reabsorption of bone by osteoclasts [55,55]. Osteoclasts have calcitonin

receptors, and CT rapidly inhibits the action of osteoclasts. They are widely used clinically to prevent and treat osteoporosis. Use of calcitonin has been found to be particularly useful in patients where estrogen therapy fails. Calcitonin reduces acute pain associated with osteoporotic fractures and has been found useful in treating chronic back pain following vertebral fractures in spinal osteoporosis. It can prevent bone loss and may be effective in preventing fractures. CT therapy results in an increase in bone mineral density and in one unblinded study it resulted in a decrease in the rate of vertebral fractures. Side effects are generally mild such as gastrointestinal, vascular and dermatologic conditions that can be treated symptomatically or by varying the dosage. It is also less effective at preventing cortical-bone loss as opposed to cancellous-bone loss in postmenopausal women.

Calcitonin has been isolated and characterized from several species including man. All CTs are 32 amino acid peptides and highly conserved molecules. Synthetic preparations of several CTs are available for clinical use. Of these porcine (pCT), human (hCT) and salmon (sCT, Salcotonin) have been synthesized according to their natural sequences whereas eel calcitonin (eCT) is available as amino-suberic derivative (ASU-eCT). Marked differences in potencies and biologic effects have been observed among all the CT species. The hypocalcemic effect and the increase of plasma cAMP are produced by all peptides in the following order: sCT > hCT > ASU-eCT [25,55]. In fact, sCT was found to be at least 40 to 50 times more potent than hCT in inhibiting osteoclastic bone reabsorption. Indeed for all calcitonins the major side effect has been flushing, caused in the following order: hCT > sCT = ASU-eCT [55].

Calcitonins have been found to be active both by parenteral administration as well as a nasal route. Side effects with nasal administration are significantly reduced over injection. Formulations for nasal administration have been prepared both for hCT and for Salcotonin and their efficacy has been extensively studied by several groups [56,57]. In established osteoporosis, nasal CT possesses a potent analgesic effect, reduces the duration of bed confinement, and decreases the number of concomitant analgesic medications. These studies suggest that administration of calcitonins by a nasal route is an attractive alternative for the treatment of post menopausal osteoporosis. Recently, a nasal formulation has been approved by the Food and Drug Administration for clinical use in humans.

8) Parathyroid hormone and its fragments

The human parathyroid hormone hPTH (1-84) and its N-terminal fragment hPTH (1-34) are promising anabolic agents for the treatment of osteoporosis [58,59]. Both stimulate cortical and trabecular bone growth in osteopenic, ovariectomized rats and in osteoporotic postmenopausal women when administered subcutaneously (SC) at lower doses [60,61]. At higher doses however, hPTH is known to stimulate reabsorption of bone. In humans, daily injections of hPTH (1-34) peptide in combination with estrogens for 12 months, holds great promise for the treatment of patients with osteoporosis who have already lost substantial amounts of spinal cancellous bone [62]. Recently

Whitefield et al. reported a new osteogenic PTH fragment, hPTH (1-31)-NH2 (ostabolin), as the minimum sequence that was as active as hPTH (1-84) in ovariectomized rats [63,64]. The truncated fragment can still activate adenylyl cyclase (AC) as effectively as rhPTH (1-84) and PTH (1-34) but, unlike the larger molecules, it can not stimulate phospholipase C-bl and thereby trigger a cascade of Ca+2- and PKC- mediated events because it lacks the critical residues, 32-34, of the phospholipase C-β1 activating domain. In ovariectomized rats (OVX) rats, daily sc injections of 0.4 to 1.6 nmol/100 g of PTH (1-31)-NH2 for 6 weeks, could greatly thicken trabeculae and increase the dry weight and calcium content of trabecular bone in the distal femurs. Thus, ostabolin may be the minimal anabolic PTH fragment, with great therapeutic potential for the treatment of osteoporosis. Recombinant hPTH (1-84), is currently in phase II clinical trials for the treatment of osteoporosis.

9) Parathyroid hormone related protein (PTHrP)

Parathyroid hormone related protein is a large polypeptide and comprises of 139, 141 or 173 amino acid residues as predicted by alternate mRNA splicing. It is produced by variety of tumors and may be the principal cause of humoral hypercalcemia of malignancy and its stimulated bone reabsorption [65,66,67]. From studies of the action of PTHrP (1-141) on populations of osteoclasts disaggregated from neonatal rat bones, it was found that rhPTHrP (1-141) had a direct effect on osteoclasts to inhibit bone reabsorption. However, studies with various fragments of PTHrP have shown that there are three distinct regions in the molecule with different biologic activity. The N-terminal fragment PTHrP (1-34), whose primary structure is quite similar to PTH (1-34), stimulated osteoclastic bone reabsorption at lower dose by acting on osteoblasts and restricting calcium by the kidney [unlike PTH (1-34)]. The central region in the PTHrP (35-;108) was found to be essential for the effect of PTHrP (1-141) in promoting placental calcium transport in the sheep [68]. The carboxyl fragment, PTHrP (107-139), was found to be a potent inhibitor of osteoclastic bone reabsorption both in vitro [70] and in vivo [69]. Indeed, there are many potential proteolytic sites within the PTHrP molecule, and there is also evidence for cleavage products in circulation. Thus osteoclast inhibitory activity is localized to a carboxyl-terminal fragment, hPTHrP (107-139).

Subsequently, Fenton et al. [71] identified a highly conserved pentapeptide corresponding to residues 107 to 111 in the C-terminal region of PTHrP (109-139) as a potent inhibitor of bone reabsorption by osteoclasts in vitro. These results were further confirmed in vivo [72], where the pentapeptide led to significant inhibition of bone reabsorption when stimulated by PTHrP (1-34). The discovery of a short peptide with such potent osteoclastic inhibitory actions lays the foundation for the development of further analogues with potential therapeutic use in disorders associated with increased osteoclastic bone reabsorption.

Recently, two groups independently studied the effects of truncated fragments: PTHrP (1-31)-NH2 and its analog RS-66271, derived from the N-terminal region of PTHrP, in the stimulation of bone growth in OVX rats.

hPTHrP (1-31)-NH2 was expected to stimulate both AC and bone growth as strongly as PTH (1-31)-NH2, due to sufficient structural similarity and location of functional domains. On the contrary, although hPTHrP (1-31)-NH2 stimulated AC and triggered a large drop in mean blood pressure in the rats as effectively as hPTH (1-31)-NH2, it failed to stimulate trabecular bone growth in OVX rats [73]. However, RS-66271, that the amino acid residues from 22 to 31 have been replaced with a sequence serving to further stabilize the low content of a-helical structure already present in PTHrP (1-34)-NH_2, exhibited a low order of activity in the in vitro studies, it exhibited increased potency in restoring the bone loss in ovariectomized, osteopenic rats, rabbits and macaques [74]. This is in striking contrast to PTHrP (1-34)-NH_2 that exhibited bone reabsorption activity at a lower dose. Thus, RS-66271 appears to be a promising therapeutic agent in the treatment of osteoporosis.

10) Insulin like growth factors

Insulin-like growth factors (IGFs) are considered to be among the most important skeletal growth factors not only because they appear to be the most abundant factors present in bone, but also because they have important actions on bone cell function. The 2 IGF genes are expressed by skeletal cells and though IGF-I and -II have similar effects on bone metabolism. IGF-I is more potent than -II [75]. IGFs have modest mitogenic activity for cells of the osteoblastic lineage, and enhance the differentiated function of the osteoblast. IGFs stimulate type I collagen synthesis and increase matrix apposition rates, independent of their mitogenic activity. In addition, IGFs inhibits collagen degradation, most likely because they inhibit the expression of interstitial collagenase by the osteoblast. These effects are critical to the formation of new bone and to the maintenance of bone matrix. Treatment of patients with IGF-I was found to increase the serum levels of type I procollagen peptide, a marker of bone formation and, in one experiment, increased the urinary excretion of collagen crosslinks, suggesting an increase in bone turnover [75]. No changes in serum levels of Ca^{2+} or calcitropin hormones were detected. Although these results are encouraging, there are potential problems with the systemic use of IGF-I. Side effects such as hypocalciurea, edema, postural hypotension and tachycardia have been reported.

Recently Jonsson et al. [76] investigated the effects of IGF-I on osteoclast recruitment and bone reabsorption in vitro. IGF-I stimulated the formation of multinucleated tartrate-resistant acid phosphatase-positive cells in murine bone marrow cultures. IGF-I had no effect by itself on ^{45}Ca-release from prelabeled neonatal mouse calvarial bones. However, it had an inhibitory effect on bone reabsorption induced by prostaglandin E2 and 1,25(OH)2D3. These findings indicate that IGF-I enhances the formation of osteoclasts-like cells in long term bone marrow cultures. In bone organ cultures, however, IGF-I has an inhibitory effect on stimulated bone reabsorption, suggesting that IGF-I inhibits existing osteoclasts and, alternatively, that IGF-I interferes with osteoblast-derived factors that stimulate existing osteoclasts. It is expected that agents that either enhance the synthesis or activity of the locally produced IGF-I in the skeleton may play a

role in the future treatment of osteoporosis.

11) Insulin

The pancreatic beta cell product insulin has long been established as an osteoblast regulator. Osteoblast-like cells have insulin receptors [77] and insulin promotes their growth [78]. In bone organ culture, insulin stimulates collagen synthesis at periphysiological hormone concentrations [79]. Earlier studies in diabetic rats though, showed that insulin treatment could bring decreased bone formation and mineralization as well as altered collagen synthesis to normal levels. The beneficial effects were attributed to correction of their severe metabolic disturbance [80,81]. Recently Cornish et al. [82], using newly developed techniques for studying the local effects of factors on bone histomorphometry, studied the effect of insulin on bone in vivo without the complicating factors of either ketosis or hypoglycemia. They observed that insulin has a direct anabolic effect on bone in vivo. All indices of bone formation measured were increased 2- to threefold in insulin-treated hemicalvariae compared with those in the noninjected hemicalvariae. These findings suggest that the direct effects of this hormone, seen in isolated osteoblast-like cells, also occur in intact bone tissue in vivo. This provides an explanation for the association between bone density and circulating insulin levels in normal postmenopausal women.

12) Osteogenic growth peptide (OGP)

A 14-amino acid peptide, called osteogenic growth peptide and identical to the C-terminus of histone H4, has been recently characterized in regenerating bone marrow. It has been identified in human as well as in animal serum, where it is present as an OGP-OGP binding protein complex [83,84]. In normal human serum, the total immunoreactive OGP content, comprised of at least 80-90% bound peptide, ranged from 480 to 4460 umol/l, several orders of magnitude higher than that of other regulatory polypeptides. The natural occurrence of OGP in man signifies its potential role in the prevention of bone loss and rescue of bone mass, especially in osteoporosis. The identity of human OGP with that of other species indicates its evolutionary conservation and thus, its biologic significance. Synthetic OGP stimulated the proliferation and alkaline phosphatase activity of osteoblastic cells in vitro. Exogenously administered OGP markedly enhances bone formation and increases trabecular bone mass in experimental animals. However, its mode of activity differs from most other osteoblast affector substances. Whereas OGP induces an acute enhancement in bone formation, factors such as prostaglandin E2 and PTH, when administered in vivo, initially stimulate osteogenic cell proliferation with a resulting delay in the promotion of bone formation. Further, the marked temporal elevation in serum OGP induced by marrow ablation, the in vivo effect of exogenously administered OGP and the in vitro data together indicate that OGP is a key factor in the mechanism of the systemic osteogenic reaction to marrow injury

13) Neuropeptides

Studies from different laboratories have suggested that bone metabolism may be influenced by the nervous system. Bone and periosteum are innervated by both sympathetic and sensory nerves. Neuropeptides, such as

vasoactive intestinal peptide (VIP), substance P (SP), neuropeptide Y (NPY) and calcitonin gene-related peptide (CGRP), are localized in nerves in bone and periosteum and have been implicated as mediators of bone formation and reabsorption that may be involved in the local regulation of bone metabolism. Recently Togari et al. [85] have successfully demonstrated the expression of mRNAs for the neuropeptide receptors in human osteoblasts and human osteogenic sarcoma cells. Thus, these active peptides, localized in nerves in bone and periosteum, provide new therapeutic approaches for the development of clinically efficacious agents for the treatment of osteoporosis.

14) Substance P (SP)

Substance P, an undecapeptide, has been found to exhibit osteogenic stimulating effects on developing bone in vitro. Using bone marrow white cells, Shih and Bernard [86] demonstrated that the addition of neurogenic SP increased the number and size of bone colonies in a dose-dependent manner. This effect can be attributed to stimulating stem cell mitosis, osteoprogenitor cell differentiation or osteoblastic activity. However, its effect in vivo has not yet been established.

15) Calcitonin gene related peptide (CGRP)

The calcitonin/CGRP multigene complex encodes a family of peptides: calcitonin, its C-terminal flanking peptide, Katcalcin, and a third peptide, CGRP. The latter is a 37-amino acid neuropeptide abundantly concentrated in the sensory nerve endings innervating bone metaphysis and periosteum. CGRP-a and -b share structural and functional homology with the CT molecules and play a local role in bone metabolism. CGRP has profound effects on calcium metabolism that are species specific [87]. It inhibits IL-1a-mediated bone reabsorption because of its direct regulation of osteoclast activity. In rats, CGRP shares the acute effects of CT at a 1000-fold higher molar concentration. The peptide causes hypocalcemia, stimulates cAMP formation in rat calvarium, inhibits reabsorption of intact bone and inhibits spreading motility and reabsorption of bone by isolated osteoclasts [94]. These effects of CGRP are probably exerted on osteoclasts via the CT receptor. In man, the infusion of CGRP does not affect plasma calcium, but the peptide causes a rise in cAMP levels in normal human osteoblasts. Valentijn et al. [88] studied the effect of CGRP in ovariectomized rats as a high bone turnover model and compared it with CT. CGRP was found to inhibit bone reabsorption but not bone formation. It was less efficient than CT in preventing bone loss, because CGRP-treated rats had a loss of 46% of cancellous bone, whereas CT-treated rats had a loss of 21%. This suggests that CGRP is either less potent than CT at inhibiting bone reabsorption or is very rapidly degraded. Structure-activity relationship studies of hCGRP are being carried out in different laboratories with a view to designing novel analogs with therapeutic potential [89,90,91].

16) Amylin

Amylin, a 37-amino acid peptide cosecreted with insulin from the beta cells of the pancreatic islets, bears 43% homology with hCGRP and 15% homology with hCT. It has been shown to have a potent hypocalcemic effect in rat and rabbit, owing to the inhibition of osteoclast-mediated bone

reabsorption. The hypocalcemic potency of amylin was found to be second only to that of CT and is 100-fold more potent than CGRP. It was found to stimulate cAMP production in osteoclast-like multinucleated cells (MNC) but only at 60-fold higher concentrations than hCT. It may be binding to both CT receptors in osteoclast-like MNC as well as to CGRP receptors in osteoblasts. In vitro, periphysiological concentration of amylin stimulated proliferation of fetal rat osteoblasts [92]. In vivo daily injections for 5 days over the calvariae of adult mice led to substantial increases in histomorphometric indices of bone formation, a reduction in bone reabsorption, and a significant increase in mineralized bone area. Equimolar doses of CT in this model produced an inhibition of bone reabsorption but no significant effect on bone area [92]. These findings support a role for amylin as a physiological regulator of bone and suggest that it should also be evaluated as a potential treatment for osteoporosis.

17) Transforming growth factor b(TGFb)

TGFb is one of the most abundantly occurring growth factors in bone matrix, most of which is in a latent form and needs to be activated to exert its biologic activity. It is presumed that osteoclasts might be playing a key role in the delivery and activation of latent TGFb, that in turn regulates the coordinated activities of osteoblast and osteoclasts in skeletal development. It has also been argued that estrogen deficiency in postmenopausal women results in the decreased production of TGFb from bone cells, and that diminished skeletal TGFb may play a role in the pathogenesis of bone loss and fractures [93,94]. In several in vitro models, TGFb has been shown to have an acute effect on bone reabsorption. In vivo studies with TGFb1 in ovariectomized rats showed a significant drop in bone reabsorption in comparison to vehicle-injected bones [95]. Similar studies were also undertaken to evaluate the in vivo effects of TGFb2 on bone and marrow cells in the OVX rat bone loss model. Ovariectomy causes a significant increase in total mononuclear marrow cells, the number of TRAP positive multinucleated cells formed in a culture of marrow cells and the number of trabecular osteoclasts and osteoblasts followed by loss of cancellous bone in the proximal tibia. TGFb2 completely prevented the increase in the number of TRAP positive multinucleated cells, and caused a small, but not statistically significant, decrease in the number of trabecular osteoclasts. However, TGFb2 had no significant effect on the number of total mononuclear marrow cells or on the loss of cancellous bone due to ovariectomy. Thus TGFβ2 may be playing a major role in the regulation of the proliferation of osteoclast progenitors in bone marrow in vivo. Recently, Boonen et al. have used recombinant TGFβ therapy in nonosteoporotic older men and found increased bone turnover and formation.

18) Bone morphogenetic proteins (BMPs)

A major advance in the understanding of bone formation has been the identification of an entirely new family of protein initiators, the bone morphogenetic proteins, that regulate cartilage and bone differentiation in vivo. These factors were originally discovered from the extract of demineralized bone and were found to induce new bone formation in

ectopic sites and, therefore, have enormous potential in bone repair. To date, 8 different BMP polypeptides, termed BMP-1 to BMP-8, have been isolated [96]. Based on the homology of the primary amino acid sequences, seven out of eight BMPs (BMP-2 to -8) have been shown to be the members of TGF-β superfamily, a large family of structurally related signaling proteins with diverse activities on cell growth and differentiation. The availability of recombinant human BMPs has laid the foundation for the cellular and molecular dissection of bone development and regeneration.

Hiraki et al. [97] studied the effects of highly purified BMP-2 and -3 on growth plate chondrocytes and osteoblastic cells in vitro. BMPs induced rapid maturation of chondrocytes at a growing stage, transformed the cells into rounded cells and induced a marked accumulation of cartilage matrix. These results suggest that BMPs have unique biological activities in vitro that lead to growth and phenotype expression of cells playing a critical role in endochondral bone formation. Similarly the effect of rhBMP-2 on osteochondrogenesis was examined in high density cultures of periosteum-derived cells, that have the potential to differentiate into bone and hypertropic cartilage in vitro. The results indicate that rhBMP-2 shortens the time course of osteogenesis and increases the amount of bone formation, whereas chondrogenesis remains unaffected [98]. Further implantation of the rBMP-2A in rats showed that a single BMP can induce bone formation in vivo. A dose response and time course study using the rat ectopic bone formation assay revealed that implantation of 0.5-1.5 μg of partially purified rBMP-2A resulted in cartilage by Day 7 and bone formation by Day 14 [99]. Among BMPs, rhBMP-7 (osteogenic protein 1, hOP-1) has been the most widely studied protein for the induction of cartilage and bone formation [100,101]. In the rat SC bone induction model, hOP-1 was capable of inducing new bone formation with a specific activity comparable to that exhibited by highly purified bovine osteogenic protein preparations [101]. Examination of the expression markers characteristic of the osteoblast phenotype showed that hOP-1 specifically stimulated the induction of alkaline phosphatase, parathyroid hormone-mediated intracellular cAMP production and osteocalcin synthesis. In long-term cultures of osteoblasts in the presence of b-glycerophosphate and L(+) ascorbate, hOP-1 markedly increased the rate of mineralization as measured by the number of mineral modules per well [101]. These studies suggest that rhBMPs have grea

In recent years much emphasis has been given on the development of a suitable delivery system for BMP to enhance its therapeutic efficacy. Use of gelatin capsules, polylactic acid-polyethylene glycol (PLA-PEG) copolymer and bovine fibrous collagen membrane as carriers for BMPs have been reported to be very promising in inducing bone formation and their applications in clinical practice are expected in the near future.

Ⅳ. Bone markers and clinical diagnosis in osteoporosis and rheumatis arthritis

Bone is continuously resorbed and formed in the process of remodeling. Osteoporosis results when bone loss is sufficient to cause an increased risk of fractures. High rates of

bone turnover can be identified by measuring biochemical byproducts released by osteoblasts and osteoclasts. The major validated biochemical markers of bone formation currently in use include the bone isoenzyme of alkaline phophatase, osteocalcin and propeptides derived from the N- or C-terminal ends of the type I procollagen molecule. The most useful markers of bone reabsorption are breakdown products of type I collagen. The most well-established of these is the measurement of hydroxyproline in collagen peptides in urine, but the assays are cumbersome. Furthermore, hydroxyproline is not specific to bone collagen and is also derived from the diet. There is, therefore, much current interest in collagen products that are more specific to bone, including galactosyl hydroxylysine, and the collagen crosslinks, pyridinoline and deoxypyridinoline. The pyridinolines and peptides derived from crosslinked regions in collagens appear to be the most promising markers of reabsorption and enable quantitative evaluation of rates of bone resumption in man. These biochemical methods are of use in the diagnosis and evaluation of bone diseases, in population studies and for monitoring responses to hormones and drugs in clinical studies. There is an increasing amount of work being devoted to the study of peptide based bone biomarkers in osteoporosis.

1) Procollagen I extension peptides

More than 90% of the organic matrix of bone is comprised of type I collagen. The precursor of collagen is procollagen from which the amino terminal (PINP) and carboxy terminal (PICP) extension peptides are proteolytically cleaved before its entry into extracellular matrix. These peptides circulate in blood where they represent useful markers of bone formation.

2) Collagen peptides

ELISAs for measuring type I collagen crossslinked N-telopeptide (NTx) and the c-telopeptide (cross laps) in urine have been developed as markers for bone reabsorption. The overall levels of these peptides were found to be markedly increased after the menopause [102]. Therefore they are excellent markers for bone reabsorption. Gertz et al. have proposed that the NTx assay could be also beneficial for monitoring changes in bone turnover in individual patients, when a 50% reduction in bone turnover is possible as a result of therapy [103].

In addition, an ELISA for serum measurement of the c-telopeptide to helix (ITCP) has been also developed. This marker was found to be enhanced in osteoporotic patients treated with anabolic steroids. In fact, it appears more of an indicator of collagen turnover rather than bone reabsorption. Recently, a new ELISA for measuring type I collagen products in serum has been reported [104The assay uses a high affinity polyclonal antibody that reacts with an isomerized form of an octapeptide present in the c-telopeptide of type I collagen. Clinical evaluations with this test suggest that it is a sensitive and specific index of bone reabsorption and can be successfully used for the clinical investigation of osteoporosis.

V. Conclusion

As outlined in this brief review, the role of structurally diverse peptides for the

management of osteoporosis is gaining significance. Several new agents have recently graduated from the laboratory to extensive clinical trials. Although these peptides provide new prospects for the prevention and treatment of osteoporosis, progress in this direction awaits identification of growth factors or analogs that will be capable of treating osteopenia disorders. In the near future, new targets for the prevention of osteoporosis are likely to be identified. This, together with other insights, will provide a foundation for the development of suitable drug for the management of osteoporosis.

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