Recent Developments in Metabolic Bone Diseases: a Gnathic Perspective

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Abstract Metabolic bone diseases often are asymptomatic and progress sub clinically. Many patients present at a late stage with catastrophic skeletal and extra skeletal complications. In this article, we provide an overview of normal bone remodeling and a synopsis of recent developments in the following conditions: osteoporosis, rickets/osteomalacia, endocrine-induced bone disease, chronic kidney disease-mineral bone disorder and Paget's disease of bone. Our discussion will emphasize the clinical and microscopic manifestations of these diseases in the jaws.

Keywords Metabolic bone disease · Histomorphometry · Bone homeostasis · Jaw bone pathology

Introduction

Skeletal changes induced by metabolic derangements often progress silently and may not be diagnosed until development of end-stage complications or failure of a clinical procedure involving bone. The frequency of radiographic examinations in dental practice and the presence of bone in biopsies of the jaws provide opportunities for the early diagnosis of metabolic diseases affecting the entire

skeleton. Here we provide a review of recent developments and advances in metabolic bone diseases, with special consideration given to characteristic clinical and microscopic findings in the jaws. Our discussion is based on current scientific evidence and the experience gained in our laboratory.

Bone Remodeling

Bone remodeling maintains serum calcium (Ca^{2+}) and phosphate (P) concentrations over a narrow range. The process occurs in small foci (bone multicellular units or BMU's), which are active for several months and exhibit alternating phases of osteoclast-mediated resorption followed by osteoblast-mediated bone formation. BMU's function within highly organized metabolic environments, referred to as BRC's (bone remodeling compartments), which is distinctly different for trabecular- and cortical bone [1]. Nuclear factor κB (NF- κB) signaling is required for RANK ligand-induced osteoclast formation [2]. The osteoblasts in the vicinity of a BMU balance their influence on osteoclasts through the release of macrophage-colony stimulating factor (CSF-1) as well as osteoprotegerin (OPG) [3–5] which inhibits osteoclast activity by acting as a decoy receptor for RANKL [6]. PTH stimulates the release of CSF by osteoblasts [7], facilitates the intestinal absorption and renal reabsorption of Ca^{2+} and is a potent stimulus for osteoclast-mediated bone resorption with subsequent mobilization of skeletal Ca^{2+} . The interaction between osteoblasts and osteoclasts is complex and influenced by several other mediators (e.g., sclerostin, calcitonin, insulin, serotonin, estrogen, leptin) and cytokines which are aptly reviewed elsewhere [1, 8]. Tartrate-resistant acid phosphatase (TRAP) released at the ruffled border

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of activated osteoclasts induces bone resorption [9] (Fig. 1a) and the elaboration of Ca^{2+} and fibroblast growth factor 23 (FGF23). The release of FGF23 by bone changes the skeleton from a target organ to an endocrine organ. Osteoclasts are active for approximately 3 months and end their cell cycle through apoptosis. The osteoclast's lacuna is restored through osteoid synthesis by osteoblasts (Fig. 1b) in which the Wnt signaling pathway plays a key role [10]. Osteoid is composed mainly of collagen type I and several non-collagenous proteins, including osteocalcin (or bone GLA-protein), osteopontin, and bone sialoproteins. At the end of the mineralization phase, alkaline phosphatase declines, the majority of osteoblasts undergo apoptosis and the remainder incorporated as osteocytes in lacunae. The initial bone deposited is of a woven type. Osteocytes regulate mineral exchange, communicate through complex interactions, and remodel woven bone into the mature lamellar type. Woven bone generally constitutes less than 10 % of bone in a microscopic section, and disturbances of lamellation are helpful in diagnosing conditions like osteogenesis imperfecta in which the maturation of osteoblasts is arrested. The mosaic pattern created by the cemental lines between the new and old bone serve as a historic record of a restored BMU.

Research Controversies and Practical Clinical Considerations

Current controversies in this research field concern the relevance of animal models for studying human disease

and the utility of crude bone examination techniques (e.g. conventional radiography, bone scintigraphy, X-ray absorptiometry). In addition, although urine and serum concentrations of the markers of bone metabolism play a role in the diagnosis, a single determination is often of limited value due to the influence of circadian rhythms, variations in the efficiency of metabolic clearing pathways, and fluctuations in volume distribution between the fluid compartments. Although quantitative bone histomorphometry has limitations such as the impact of anti-remodeling agents on bone metabolism, interpretative errors and sampling pitfalls [11], it is the diagnostic tool of choice in our laboratory, as it provides reproducible analytical capability at cellular level. This method entails *in vivo* bone labelling by administration of two cycles of tetracycline (a calcium substitute) at 14-day intervals [12]. Non-demineralized sections are prepared of trans cortical bone biopsies, appropriately stained, and subjected to histomorphometry. The volume of osteoid, indices of resorption, intensity, and distance between the fluorescence of tetracycline-labeled mineralization lines are measured for both cortical and trabecular bone, and compared to standardized reference ranges [13]. Correlation of the histomorphometric, biochemical, clinical, and imaging findings aids in determining the diagnosis and appropriate management.

Although the outline and total volume of bone, resorptive facets and microscopic detail of associated cells are retained in routine demineralized bone biopsies, the ability to determine osteoid volumes and mineralization parameters are sacrificed. H&E stained routine biopsies, however,

Fig. 1 Bone multicellular unit. **a** Osteoclasts (arrows) in a shallow lacuna. Note the osteoclast's ruffled border on the surface of the bone. **b** Cemental line between bone and osteoid (black arrows) and the

plump osteoblasts (white bordered arrows), which actively restore the osteoclast lacuna (H&E stain, non-demineralized sections, magnification $\times 1000$)

Fig. 2 Osteoporosis. **a** Trabecular bone demonstrating thinning and disappearance of individual units (*arrows*) (Bar = 1 mm). **b**. Trans cortical biopsy of the iliac crest showing a reduced trabecular bone

volume (16 %, $n = 22,5$ %; SD3.5 %) and a mean cortical width of 620 μ ($n = 909$ μ ; SD 98 μ), which confirms osteoporosis (H&E stain, $\times 40$ magnification)

provide valuable information on bone volumes, resorptive activities and bone marrow changes, which indicate disease.

Osteoporosis

After peak skeletal mass is reached between 25 and 35 years of age [14], the mild progressive bone loss of normal aging is related to preserved osteoclastic and decreased osteoblastic activities. This physiological decline in bone mass is exacerbated by several genetic and lifestyle factors which lead to a reduction of bone beyond normal age matched norms. Osteoporosis is the state in which the cortical and/or trabecular compartments are qualitatively normal but reduced in volume (Fig. 2). Major reasons that females are at increased risk for osteoporosis include a lower peak bone mass in young adulthood, longer life expectancy compared to males, menopause-related estrogen deficiency, and lifestyle factors (e.g., vegetarian and high fiber diets that retain dietary Ca^{2+} in the gastrointestinal tract) [15, 16].

Mandibular cortical thickness less than 3 mm (as measured below the mental foramen on a panoramic dental radiograph) and cortical erosions are convincing predictors for osteoporosis and warrant referral for further examination [17, 18]. The effect of osteoporosis on periodontal disease is inconclusive, although a significant association with tooth loss has been reported in some communities [19]. Osteoporosis is not an absolute contraindication for placing dental implants [20]; however, patients receiving anti-resorptive drugs, radiotherapy and smokers should be

informed of the increased risk of implant failure [21]. Current trends in implant research focus on the coating of dental implants with calcium phosphate and organic biomolecules that enhance bone formation at the bone-implant interface [22]. Osteoporosis also has been reported to accelerate orthodontic tooth movement [23].

For patients with osteoporosis, studies of rare genetic bone disorders are unlocking possibilities for improving bone mass by simulation of gain-of-function mutations. For example, osteosclerosis (OMIM: 144750) results from mutation of *LRP5* (the low density lipoprotein receptor-related protein 5 gene) and up-regulation of bone formation. Patients present with a high bone mass, elongated mandible, midline torus palatinus, and thickened cranial plates [24]. The canonical Wnt signaling pathway is now recognized as a target for therapeutic intervention for millions of patients with osteoporosis [25]. The importance of Wnt signaling during bone formation is illustrated by van Buchems disease (idiopathic radiodensities in the jaw bones) and sclerosteosis, which result from mutations of the gene coding for the Wnt antagonists sclerostin (SOST). Neutralization of these antagonists enhances bone formation and could be exploited to manipulate skeletal bone mass. In addition, insertion mutations of the RANKL signaling peptide have been demonstrated in Paget-like disorders, expansive skeletal hypophosphatasia, familial expansile osteolysis, and osteopetrosis [26, 27]. Another target for therapeutic intervention in osteolytic disease is NF- κ B, which is the core cytokine for inflammatory osteolysis [1] and which could be modulated through immunological and pharmacological mechanisms to establish an anabolic bone phenotype.

Fig. 3 Rickets. **a** Low power photomicrograph showing wide osteoid seams around the mineralized component of the trabecula. The arrow indicates a focus of resorption of exposed mineralized bone (non-demineralized section, Picrosirius stain, magnification $\times 100$). **b** Narrow mineralization front (between arrows) indicating absence of mineralization activity in osteoid. **c** Broad mineralization front (between arrows) characteristic of enhanced mineralization (black

star—osteoid, white star—mineralized bone, Von Kossa stain, non-demineralized sections, $\times 200$). **d** Two fluorescent tetracycline lines in labelled bone (arrows) reflect a calcification rate of 0.6 microns per day and the linear extension of the labelled osteoid translates into adequate mineralization activity and successful management (unstained, non-demineralized section viewed with UV light, magnification $\times 400$)

Disorders that Disrupt the Provision of Metabolites

Bone mineralization requires a constant Ca^{2+} supply, which depends in part on the availability of vitamin D_3 , dietary calcium, and an intact intestinal mucosa. Among our patients, hypocalcemia and decreased bone mineralization in those on supplemented diets often indicate intestinal malabsorption associated with long term antibiotic or corticoid therapy, intestinal infections (parasitic infestation, tuberculosis, tropical sprue or Whipple's disease), Crohn's disease, and celiac disease. Bone marrow eosinophilia may be a clue for underlying parasitic intestinal infestation or celiac disease.

Insufficient bone mineralization due to various mechanisms that result in hypocalcemia, hypophosphatemia, or direct inhibition of the mineralization process is referred to as rickets in the growing and osteomalacia in the mature skeleton. In both conditions, the volume of osteoid is increased, Ca^{2+} incorporation minimal, unlike osteoporosis in which the bone is normal but quantitatively reduced. In the late stage of rickets and osteomalacia, extensive coverage of bone by osteoid is a prominent feature (Fig. 3a). If management fails, the osteoid barrier does not mineralize (Fig. 3b) and prevents mobilization of skeletal Ca^{2+} ,

precipitating a fatal hypocalcaemia. With successful mineral supplementation, bone histomorphometry shows quantifiable changes (Fig. 3c, d). Microscopic examination of the developing dentition in rickets shows widening of the predentin zone (which correlates radiographically with generalized enlargement of the pulpal cavities), dentinal vascular inclusions, and enamel hypoplasia. In total starvation (kwashiorkor–marasmus syndrome), the bone lacks osteoid [28], a state responding poorly to mineral supplementation.

Endocrine-Induced Bone Disease

Hyperparathyroidism may be primary or result secondarily from metabolic changes associated with rickets, osteomalacia, and chronic kidney disease. Microscopically, there are an increased number of osteoclasts per linear millimeter bone surface; these osteoclasts mobilize Ca^{2+} . However, serum Ca^{2+} , P, and alkaline phosphatase concentrations are poor indicators of a mild to moderate deficiency state [29]. Consequently, subclinical disease detection requires bone biopsy with non-demineralized sections subjected to histomorphometric analyses. The release of FGF23 during

Fig. 4 Tunneling resorption directly after suspension of secondary hyperparathyroidism and before osteoblast differentiation. Note the nuclear debris generated by apoptosis of osteoclasts and the fibrous marrow scar, which indicate past osteoclast activity (H&E, magnification $\times 400$)

bone resorption induces catabolism of vit D₂ and regulates phosphate metabolism by inhibiting its renal tubular reabsorption [30]. The penetrating mode of resorption is aptly described as “tunneling” and is pathognomonic for hyperparathyroidism. Apoptosis of osteoclasts (Fig. 4) with osteoblast differentiation and filling of the facet with bone indicate normalization of parathyroid activity through the provision of metabolically active Ca²⁺. Residual bone marrow fibrous scars provide long-term evidence of past osteoclast activity.

The skeleton plays an important role in energy metabolism through the intestinal endocrine pathways. Manipulation of gut-derived hormones, such as serotonin, provides a novel approach to the treatment of osteoporosis [31]. Obesity has traditionally been thought to exert a protective influence on bone, possibly related to the inverse relationship between osteocalcin and insulin concentrations [32] and the release of leptin by adipocytes. In a recent study it was found that osteoporosis and related fractures is an underestimated and clinically significant problem in patients with diabetes [33]. Endogenous Cushing’s syndrome in children is reported to be associated with normal and even advanced skeletal maturation. The latter is related to obesity, insulin resistance, aromatization, and elevated androgen levels [34].

Chronic Kidney Disease–Mineral Bone Disorder (CKD–MBD)

Chronic kidney disease is a growing international health problem, and is linked to CKD–MBD, a systemic disorder

Fig. 5 Chronic kidney disease. Cropped panoramic radiograph showing loss of the lamina dura around teeth and reduction in bone manifesting as trabecular effacement and severe cortical thinning. Note the metastatic soft tissue calcification (white arrow)

of mineral metabolism and skeletal and soft tissue complications. It is characterized by abnormal serum Ca²⁺, P, PTH, or vit D concentrations, abnormalities in bone turnover, mineralization and volume, and calcification of the vasculature or other soft tissues [35, 36]. CKD–MBD results from impaired hydroxylation of vit D with decreased renal retention and reduced intestinal absorption of Ca²⁺. The resulting hypocalcemia induces the release of PTH with tunneling resorption of bone and subsequent elaboration of FGF23. The latter inhibits parathyroid function, induces phosphaturia and suppresses the activation of vit D [37]. Administration of antibodies against FGF23 in rats with chronic kidney disease has a positive influence on the skeleton through normalization of parathyroid functions and increase of vit D. However the increased mortality through the induction of vascular calcifications limits the therapeutic use of anti-FGF23 antibodies in humans [38]. The earliest radiographic signs of jaw bone involvement in CKD–MBD are thinning of the cortices and loss of the lamina dura (Fig. 5). The resorbed lamellar bone is replaced by fibrous connective tissue, a microscopic feature described as “osteitis fibrosa”. This finding is histopathologically similar to fibrous dysplasia but can be distinguished by correlation with the clinical and biochemical findings. The non-expansive and expansive lesions that may develop are referred to as “osteitis fibrosa cystica” and “brown tumor of hyperparathyroidism,” respectively. The term “uremic leontiasis ossea” describes a small cohort of renal patients with enlargement of the facial bones and a ground-glass radiographic appearance

mimicking fibrous dysplasia and Paget's disease. Bone scintigraphy is of limited value in distinguishing between these conditions as all three manifests with increased tracer uptake. Prolonged parathyroid stimulation may lead to tertiary hyperparathyroidism, a state in which PTH is released irrespective of a correction of blood Ca^{2+} . Tertiary hyperparathyroidism is characterized by a high concentration of PTH, hypercalcemia with metastatic soft tissue calcifications, and accelerated skeletal bone loss. It is refractory to conservative management and may require parathyroidectomy to correct. Metastatic calcification of arterial walls secondary to chronic hypercalcemia may manifest on dental radiographs [39]. Such findings are important predictors of cardiovascular impairment due to coronary and heart valve calcifications, which account for more than half of deaths among patients on long-term renal dialysis [40].

Recent Developments in Paget's Disease

The only mutation linked to Paget's disease is sequestosome-1 (SQSTM1) which encodes p62 protein. This leads to cytokine NF- κ B activation in osteoclasts [41, 42], RANKL-induced osteoclast differentiation, and bone resorption. NF- κ B activity is the core of the inflammatory response and key to inflammatory mediated osteolysis [43]. This genetic mutation alone is however insufficient to cause disease and other environmental factors, such as chronic measles virus infection, have been implicated in the pagetic osteoclast phenotype. This phenotype is characterized by increased sensitivities to vit D₃ and RANKL and increased expression of IL-6 and other transcription factors [44–46]. In addition to NF- κ B activation, SQSTM1/p62 is a regulator of ubiquitinated protein metabolism via autophagy and the proteasome system [41]. Knowledge on the molecular link between apoptosis and autophagia through SQSTM1/p62, which mediate cell survival signaling, is a field that is rapidly advancing. The success achieved in the treatment of a rare form of Paget's disease with RANKL antibody (denosumab) is promising [47]. A greater understanding of the biochemical pathways in Paget's disease will undoubtedly lead to the development of more patented drugs that target skeletal osteolysis. The role of bisphosphonates in the treatment of Paget's disease is covered elsewhere in this edition.

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