

Today's understanding about bone aging

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SUMMARY

Patient's age is an important factor in orthodontic treatment. There are many studies about bone aging from the aspects of osteoporosis and other bone diseases. Both, aging and osteoporosis are associated problems and have a great importance in relation to their incidence. But not only osteoporosis or other bone related diseases are issue for many studies, because the knowledge of bone pathophysiology and diagnostics with signaling molecules makes it possible to predict treatment outcome and specific cell targeted pharmacology. In recent years immunohistological studies had become very popular in all fields of medicine including orthodontics, too. The question we tried to answer in this literature mini-review was - what kind of immunohistological studies were done from the aspect of bone aging in relation to patient's age and orthodontic treatment?

Search on Cochrane Library, PubMed, Science Direct, and DynaMed data bases by keywords: Alveolar bone aging, RANK, RANKL, OPG, MMP-1, MMP-8, IL-1, IL-6, TNF- α , TNF- β , and BM, resulted with 147 full-text articles; from them 90 met the criteria, 30 were reviews, and only in 22 articles from 60 bone aging from the aspect of signaling molecules were discussed. Interestingly, only 2 articles (Cei 2006 and Zhang 2003) were related to alveolar bone, and none studied it from the orthodontic point of view. Patient's age plays an important role in orthodontic treatment, because of the bone response to mechanical loading. It is in accordance to clinical studies, or alike, that tooth movement in younger patient occurs much faster than in adults, but the question which factors are responsible for the process still remains.

Key words: Alveolar bone aging, RANK, RANKL, OPG, MMP-1, MMP-8, IL-1, IL-6, TNF- α , TNF- β , BMP.

INTRODUCTION

Cellular aging is a process involving progressive loss of genetic materials, accumulation of metabolic wastes, activation of aging genes and deactivation of longevity genes. In all somatic cells of mammals occurs such cellular effects as cessation of proliferation, loss of normal function, and initiation of apoptosis, commonly expressing in aging of skin, immune disorders (such as AIDS, diabetes, macular degeneration), Alzheimer's disease, atherosclerosis, and muscular dystrophy. While studies on progressive accumulation of deleterious materials in cells have been one main focus of aging studies in the past decades, searching for genes switched on or

off by mutation and deletion during aging has never stopped [11].

Aging is associated with marked changes in multiple organ systems, including bone. In humans, bone mineral density peaks between ten and nineteen years of age, with continued increase in bone mineral content until thirty to thirty-five years of age [8].

Different metabolic parameters also have been studied in the liver by using primary cultures of hepatocytes from aged rats, such as ATP (adenosine triphosphate) production and oxidative stress markers. A reduction of the former and an increase of the later have been detected with aging [19].

Once the adult's skeleton has been formed by modeling, the formation of new bone at new sites; this process becomes relatively unimportant; and remodeling or the removal and replacement of bone at the same site, predominates. Remodeling is most active in trabecular bone, where Howship's lacunae are formed by osteoclastic bone resorption and replaced by osteoblastic bone formation. The same process occurs more slowly in the cortex, and is known as

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Haversian remodeling; nevertheless, most cortical bone in adults is composed of cylindrical osteons formed by this process [16].

Aging is thought to be a contributing factor compromising the regenerative potential of bone. An age-related decrease in the number of osteogenic progenitor cells seen in animal models and in man may be one of the underlying mechanisms. In vitro findings based on explanted cultures from rabbit periosteum showed that the cambium layer, as well, as the potential of the cells to differentiate into chondrocytes are diminished with increasing age. In addition, the percentage of osteogenic cells within the bone marrow decreases with age. This may explain why the osteogenic potential of bone marrow samples from adult animals is poorer than that of marrow preparations derived from young littermates. The lower number of osteogenic cells at sites of bone regeneration and the impaired capacity of blood vessel formation are causal factors that can provide information about the mechanisms of compromised bone regeneration in elderly individuals [4].

Although there is thought to be a period of stability after completion of growth, during which time there is neither gain nor loss of skeletal mass from any surface of bone, ageing probably begins when growth ceases. BMD (bone mineral density) decreases at the spine and proximal femur in women before menopause. Bone is lost during the early adult years in men and in women because negative basic multicellular unit balance may begin at this time, in the third decade, well before menopause in women. The negative balance probably is the result of an early reduction in bone formation within each individual unit, and not due to an increase in the focal resorptive removal of bone. The hormonal and cellular factors responsible for the fall in bone formation early in life are unknown. Whether loss of bone is an appropriate response to reduced loading in a less active person, or an abnormality produced by reduced osteoblast lifespan, increased osteoclast lifespan, or abnormal osteocyte mechanical signaling is uncertain, but the effect is the same – bone loss and structural damage [17].

Although there is not a single definitive biological marker that can be used for quantifying the rate of aging, the change of aging rate in mice can be revealed by quantitative measurement of a group of aging-related traits. Lifespan unquestionably is the most important trait reflecting the rate of aging [21].

In the period from childhood to adulthood, the bone formation activity is higher than bone resorption activity, which results in a net increase of bone mass. At advanced periods of age, or particular periods of

age, the balance tips towards bone resorption, resulting in a net loss of bone mass. This makes the bone mass as a valuable marker reflecting the aging [21].

OSTEOGENESIS

Bone is a dynamic multifunctional organ that is comprised of a structural framework of calcified matrix containing populations of many cell types including chondrocytes, osteoblasts, osteocytes, osteoclasts, endothelial cells, monocytes, macrophages, lymphocytes, and hematopoietic cells. Bone tissue is constantly being remodeled throughout life in response to hormonal signals, paracrine and autocrine factors, and physical stresses. The structure of bone is formed, maintained and reformed by the collective action of cells that produce and mineralize bone matrix, and degrades it. These cells are termed osteoblasts and osteoclasts [7].

Far from the rigid, static structure that bone might be envisioned to be, the skeleton is in a state of constant degradation and reconstruction. Two of the major cell types of bone, osteoblasts and osteoclasts, are responsible for this continuous remodeling [18].

Osteoblasts are mononucleated cells originated from mesenchymal stem cells. At bone formation sites they produce and actively secrete osteoid consisting mainly of type I collagen and other proteins. Osteoid forms the bone matrix and under normal conditions is mineralized with hydroxyapatite to form the structural framework of bone. Osteoblasts maintain a high activity alkaline phosphatase and produce a number of regulatory effectors including prostaglandins, cytokines and growth factors stimulating bone formation or resorption [7].

Osteoclasts are the cells responsible for bone resorption. They are large multinucleated cells formed by the fusion of mononuclear hematopoietic precursors brought to the bone through the vasculature. Active osteoclasts reside on the mineralized surface of the bone where they produce and release lysosomal enzymes, protons, and free radicals into the space in contact with the bone. These products dissolve the mineral and degrade the bone matrix. Osteoclastogenesis is regulated by a number of cytokines, growth factors, and eicosanoids [7].

Usually the amount of bone removed by the osteoclasts is equal to the amount of bone formed by the osteoblasts and persistent bone mass is maintained. However, this is not the case during aging. Increased bone resorption and/or decreased bone formation can lead to the bone loss. Based on a number of histomorphometric studies performed on iliac crest biopsies, a decrease in bone formation seems to be the principal

pathophysiological mechanism responsible for age-related decreased bone mass [3].

Osteocytes are the most abundant and evenly distributed, longest-living, and best-connected cell types in the mineralized matrix. However, it is the canicular system through which these osteocytes communicate. Radiating dendritic processes from each osteocyte are extensive and run through the canaliculi, similar to dendritic processes in other systems. Solute transport through the bone lacunar–canalicular system is believed to be essential for osteocytes' survival and function. However, this is not the only method of signaling these cells utilize. Osteocyte apoptosis can influence the mechanosensory function of the osteocyte network. There are not enough data demonstrating the relationship between mechanical loading and sensing of these cells, it is thought that through interstitial fluid osteocyte activity is triggered and also cell-signaling molecules and nutrient waste products. The highly interconnective system appears to be one reason why osteocytes are responsive to mechanical strain, as well as translating that strain accordingly to the intensity of strain signals. Mechanical strain is translated *in vivo* either as deformation of the extracellular matrix or as fluid shear stress along the cells indicating that osteocytes have close contact with the bone matrix. This mechanical sensor ability also has been indicated in the mechanosensing process, where osteocytes directly sense the deformation of the substrate they are attached with. Osteocytes make contact with the extracellular matrix through attachment points that colocalize with vinculin. This connection is important in translating mechanical strain from extracellular signals into intracellular messages [6].

BONE AGING AND OSTEOPOROSIS

Human aging is associated with bone loss leading to bone fragility and increased risk of fractures and osteoporosis. Osteoporosis is one of the most prevalent and serious diseases, it affects the elderly population and is a significant problem of the public health. The cellular and molecular causes of age-related bone loss are currently intensive topics of investigation with the aim of identifying new approaches to prevent and treat osteoporotic bone loss [3].

The usual description of the pathogenesis of osteoporosis and fragility fractures is that there are four major elements.

1. Genetic and lifestyle factors that influence the ability to achieve peak bone mass and strength during childhood and adolescence. A large number of polymorphisms of genes that can influence bone

mass and strength have been identified now, but with the exception of a few single gene disorders such as osteogenesis imperfecta and the osteoporosis-pseudoglioma syndrome, the individual genetic differences generally contribute only a small amount of variation. Clearly osteoporosis is a polygenic disorder that may involve hundreds of different genetic contributions. There is epidemiologic evidence for substantial effects of nutrition and lifestyle on peak bone mass and on fracture risk, not only during childhood and adolescence, but even during gestation [2].

2. Fast loss of the bone mass and microarchitectural deterioration due to increased bone resorption occurs at the menopause due to estrogen deficiency. In men deficiency of estrogen and androgen also may contribute with aging. In older individuals, decreased intakes of calcium, low levels of vitamin D, and secondary hyperparathyroidism are important factors. While there are some differences in the skeletal effects of estrogen deficiency and calcium and vitamin D deficiency, the overlap is sufficient so that we no longer attempt to distinguish between “postmenopausal” and “senile” osteoporosis.

3. An inadequate bone formation occurs during remodeling. The process may begin even in very young people; and presumably involve a failure of cells of the osteoblastic lineage to replicate, differentiate and form a mineralized matrix as effectively as they did in younger individuals. Deficiency of nutrition and growth factors have been implicated but their relative importance is not known. In addition, there may be accelerated programmed cell death – apoptosis of osteoblasts and osteocytes with aging. This is known to occur when glucocorticoids are administered or when growth factors and their signal transduction pathways are impaired. Estrogen deficiency may also play a role in the impairment of bone formation. Thus absolute rates of bone formation are increased at the menopause due to the increase in the number of remodeling sites, there is an imbalance, with more resorption than formation, leading to rapid (resulting with following/forthcoming) bone loss.

4. The risk of falling may be increased by neuromuscular impairment or drugs, that cause hypotension or impair balance. Immobilization can lead to both through impaired neuromuscular function and also to increased bone resorption and decreased bone formation [16].

In the modern era, many biologic functions acquired during evolution have become maladaptive. This shift is in part attributable to dramatic changes in our living environment as well, as the sudden increase of human lifespan during the past century. In majority of western cultures, people are living well

into post-reproductive senescence. Obesity, diabetes, Alzheimer's disease, and atherosclerosis are examples of diseases of modernity that are attributable to modern life circumstances or that are unmasked during senility, an evolutionarily naive life epoch. The emergence of osteoporosis as a modern disease may be an example of this phenomenon [21]. According to this view an intervention to prevent their formation by the administration of antioxidants constitutes a major therapy for the amelioration of the aging process and age-associated diseases [12].

Despite all the pathogenetic mechanisms mentioned above, skeletal tissue remains viable, metabolically active, and responsive to mechanical forces, hormones and other regulatory factors during the lifetime. Hence it is useful to assess the factors that result in impaired cellular function the skeleton and to attempt reverse them [17].

CYTOKINES, GROWTH AND APOPTOTIC FACTORS

The remodeling process involves four following steps: activation, resorption, reversal, and formation.

1. Activation. The initiation of osteoclastic bone resorption depends on the interaction between cells of the osteoblastic and hematopoietic lineages [17]. A progressive reduction of hematopoietic tissue mass is observed with aging. The great majority of bone marrow areas show hematopoietic activity at birth, but starting from childhood a progressive fatty replacement of active marrow areas in the long bones takes place. These findings suggest that aging is associated with changes in the dynamics of hemopoietic stem and progenitor cell compartments which, although functionally irrelevant in healthy individuals, may influence the bone marrow response to pathologic events [15]. Recent studies analyzed human BMSCs (blood mesenchymal stem cells) stem/progenitor characteristics, i.e. the ability to retain the differentiation potential, during *in vitro* culture expansion. The results convinced that, as a population, BMSCs progressively lost their differentiation potential *in vitro* and bone-forming efficiency *in vivo* during the expansion period [13]. In addition, although normal fibroblasts function to maintain the intercellular matrix, senescent fibroblasts secrete collagenase and other matrix-destroying enzymes, leading to altered tissue integrity [5]. Fibroblast growth factor-23 (FGF23) was first discovered as an important factor for mineral homeostasis [9]. Osteoblastic cells can activate hematopoietic cells to differentiate into bone resorbing osteoclasts through the production of macrophage colony stimulating factor (M-CSF) and

receptor activator of NFB ligand (RANKL). Lymphocytes and malignant cells may also produce M-CSF and RANKL, and this may be the cause of excessive resorption in inflammation and cancer. Lining cells of osteoblastic origin on the bone surface may also play a role by secreting proteolytic enzymes that remove a protein layer which normally covers the mineralized matrix.

2. Resorption. Osteoclasts bind to the mineralized matrix through vitronectin receptors and then form their resorbing apparatus consisting of a ruffled border, which secretes hydrogen ions and matrix degrading enzymes, particularly Cathepsin K, in a compartment that is separated from the extracellular fluid by a sealing zone. This permits the establishment of a low pH and a high concentration of enzymes that can degrade collagen quite effectively at that low pH. In addition to this cellular compartmentalization there may be a compartmentalization of the entire bone remodeling unit because the lining cells remain intact above the osteoclastic resorption and osteoblastic formation sites. This might allow for growth factors released from osteoclasts or matrix during resorption to remain for longer and at more effective concentrations during the next phase.

3. Reversal. After osteoclastic resorption is complete there is a reversal phase during which mononuclear cells, which may be either of the mesenchymal or hematopoietic lineage, complete the removal of matrix and prepare the bone for formation by laying down a "cement line" of non-collagen proteins.

4. Formation. In the formation phase osteoblasts lay down matrix, which becomes gradually mineralized. As each layer of osteoblasts forms its assigned amount of matrix it either undergoes apoptosis, or becomes buried in the matrix as an osteocyte. As this process of replacing the resorption cavity is completed, some of the osteoblasts may remain on the surface as lining cells [15].

The role of osteoclasts in causing bone loss has been studied extensively by many investigators and several advances have been made recently. Only a few years ago the immune system was linked to bone loss and the effects of estrogen in bone metabolism were first focused on antagonism of pro-inflammatory cytokines such as IL-1 (interleukin -1), IL-6, TNF- β (tumor necrosis factor - β), GM-CSF, and prostaglandin E2. These cytokines and prostaglandins were found to increase osteoclast proliferation, but estrogens and TGF- β were found to decrease the production of these cytokines and inhibit osteoclast activation and bone resorption [7]. Few studies have examined the age-related changes in bone composition that may affect the functions of the osteoblastic cells. Bone-matrix

levels of insulin-like growth factor (IGF)-I and transforming growth factor (TGF)- β determined in bone biopsies obtained from donors of various ages, were found to decrease with aging. Both IGF-I and TGF- β are known to be an important factors controlling human osteoblast cell proliferation and functions [1]. Involvement of IGF-1 in the control of mammalian aging was first deduced from increased longevity of hypopituitary, growth hormone (GH)-deficient, and GH-resistant mice in which suppression of hepatic IGF-1 expression and the resulting in severe reduction of peripheral IGF-1 levels are prominent shared phenotypic characteristics [2]. Also, recent molecular approaches have rapidly advanced the field by the discovery and extensive characterization of three new cytokine systems of the TNF family. They have been found to regulate proliferation, differentiation, fusion, activation, and apoptosis of osteoclasts. RANKL is naturally inhibited by osteoprotegerin and is important in determining the balance of osteoclast activity [6]. This system is comprised of a ligand (RANKL) and also its specific receptor (RANK), as well as decoy receptors (OPG). In the case of RANKL, three distinct variants have been identified: (1) a cell membrane bound variant, (2) a soluble form, and (3) another secreted form produced by activated T cells, which is found to cause rapid bone loss by increasing the activity of osteoclasts. This is further supported by showing that RANKL gene deletion prevents bone loss and causes increased bone mass or osteopetrosis [7].

Osteoclast maturation requires stimulation by RANKL expressed on osteoblasts, and cognate interaction mediated by firm adhesion via inter-cellular adhesion molecule (ICAM)-1. Pro-inflammatory cytokines such as IL-1 and TNF- β favour bone resorption via the induction of RANKL and ICAM-1 on osteoblasts. These inflammatory signals originate from the immune system. Ageing is accompanied by increased TNF- β , IL-1, RANKL and MCSF expression and expansion of the osteoclast precursor pool. As a consequence, there is an increased stromal/osteoblastic cell-induced osteoclastogenesis during the aging [13].

Bone morphogenetic proteins (BMPs) are morphogens; growth factors and cytokines that belong to the transforming growth factor beta (TGF β) superfamily and having a wide range of effects on different cell types. Originally identified as protein factors able to induce new bone formation ectopically and in vivo; their roles in bone and cartilage development have been studied extensively. However, their roles in development, postnatal growth, homeostasis, and diseases extend far beyond the skeletal tissues. [14] Bertone and coworkers (2004) stated that, BMP-6

is thought to be a potent regulator of osteogenic differentiation and is being examined for potential therapeutic uses in bone tissue engineering [4].

The Cbfa1 (core binding factor alfa1) protein was first identified as the nuclear protein that bind to an osteoblast specific cis-acting element that activates the expression of Osteocalcin, an osteoblast-specific gene. Osteocalcin is noncollagenous bone matrix protein that binds to calcium and regulates bone formation and mineralization [22].

The newly emerging paradigm in the rapidly developing field of 'osteimmunology' is that recently activated T cells produce several cytokine-like factors, such as receptor activator of NF κ B ligand ('RANKL') and TNF- β that exert a dynamic effect on the activity of osteoclasts, the bone resorbing cells [5].

In the complex scenario of osteoimmunology, the T lymphocyte has the main role. The skeleton is physiologically in a state of dynamic equilibrium between a new bone formation mediated by osteoblasts and a resorption mediated by osteoclasts. Both these processes are finely tuned by cytokines and growth factors [13].

It is well known that calcium supplements prevent calcium loss in the bone due to estrogen deficiency, because of the variable responses to this therapy, several new therapeutic approaches are well underway yet. In particular, therapies designed to modulate osteoclast activity such as bisphosphonates and calcitonin, as well as to block signaling of the osteoprotegerin (OPG)/receptor activator of nuclear factor- κ B ligand (RANKL)/RANK pathway, etc. are being tried [7].

Emerging molecular evidence suggests that inflammation also exerts significant influence on bone turnover. Numerous cytokines and growth factors have been implicated in the regulation of osteoblasts and osteoclasts. IL-1, IL-6, TNF-, GM-CSF, MCSF, and PGE2 have been shown to increase bone resorption through indirect or direct modulation of osteoclasts. TNF related cytokines have been implicated as mediators in the final effectors of cytokine-mediated bone resorption. Receptor activator of nuclear factor κ B ligand (RANKL), osteoprotegerin (OPG), and receptor activator of nuclear factor κ B (RANK) have been implicated in osteoclast activity in conjunction with estrogen, 1,25(OH) $_2$ D $_3$, and parathyroid hormone [20].

Apoptosis is a genetically programmed mechanism controlling cell death. Two well-documented apoptotic paradigms are the extrinsic and intrinsic pathways of apoptosis. The extrinsic pathway, exemplified by Fas ligand-induced apoptosis, is achieved by sequential activation of initiator and executioner caspases without the involvement of mitochondria.

The intrinsic pathway is mediated by mitochondria, which propagate apoptotic signals by releasing the proapoptotic factors such as cytochrome c that activate downstream executioner caspases [21].

TNF- α also induces osteoblast apoptosis, decreasing bone formation. Circulating osteoclast precursors secrete inflammatory cytokines amplifying inflammatory circuits at a systemic level [13]. TNF- α exert a dynamic effect on the activity of osteoclasts, the bone resorbing cells [5] and is closely linked to the activation of osteoclasts, together with prostaglandins is found to accelerate bone loss [7].

Aging is associated with the phenotypic changes in the T-cell compartment and with a decline in T1 immunity [10] and as a result of imbalanced physiological functions. Both, the decrease and the increase of regenerative functions may contribute to/with the aging phenotype. In this regard, the bone represents an interesting example. In animals, during all period of life, the bone mass is maintained dynamically by the resorption of existing bones and the formation of new ones. With the progress of aging, the activity of bone resorption surpasses the activity of bone formation, which results in aged bone phenotypes such as osteoporosis and kyphosis. Recent studies state that the inhibition of caspase-2 activity in primary osteoclasts significantly increased the viability of these cells [21].

CONCLUSIONS

It is great part of interest to identify the factor or factors responsible for impaired osteoblast differentiation with aging. It is plausible that several circulated factors will be responsible for these effects since aging is associated with multitudes of changes in serum levels of hormones, growth factors, cytokines, and other serum components known to exert significant biological effects on osteoblastic cells. For example, insulin-like growth factor-1 (IGF-I), transforming growth factor β (TGF- β), interleukin (IL)-1, IL-6, and tumor necrosis factor α (TNF α) among other factors that are also known to change with aging in the bone microenvironment [1]

Many studies were done on animals and that is the reason why data cannot be fully contributed to human beings. Most of them were focused on osteoporosis in relation to bone aging. There are none studies done in orthodontics to alveolar bone and bone aging with immunohistological methods. It remains largely unknown, what kind of molecules can predict possible tooth movement speed when orthodontic force will be applied and what molecules are age-specific without orthodontic force application. In future, that will help to use cell targeted drugs to improve orthodontic treatment time and outcome.

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