Oxidative stress as a possible therapeutic target for osteoporosis associated with aging

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Correspondence: Sergio Portal Núñez - Área de Reumatología y Metabolismo Óseo - Instituto de Investigación Sanitaria-Fundación Jiménez Díaz - Avda. Reyes Católicos, 2 - 28040 Madrid (Spain)
e-mail: sportal@fjd.es

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Summary
Senile or involutional osteoporosis is a major problem in the developed world. Recent studies point to increased oxidative stress associated with aging, whether biological or chronological, as an important factor in its development. In this review paper, we focus on bone tissue disorders related to aging, the source of oxidative stress and negative influence on bone tissue. Finally, we consider the potential oxidative stress therapies currently being developed for this disease.

Key words: oxidative stress, osteoporosis, aging, fragility.
Introduction
The aging population in developed nations has led to an increase in the prevalence and incidence of osteoporosis. An estimated 200 million people suffer with this condition worldwide.

Defined as a decrease in bone mass and quality that increases the risk of fracture, osteoporosis is closely related to aging. Although the factors involved have not been fully identified, those associated with involutional osteoporosis include estrogen after menopause, glucocorticoid deficit associated with involutional osteoporosis include estrogen after menopause, glucocorticoid deficit and rhematoid arthritis. However, as in humans, rodents also suffer with this condition worldwide.

Bone disorders associated with aging
Bone tissue undergoes a continuous remodeling process, with considerable regenerative capacity and adaptation to physiological changes. This process takes place in so-called bone remodeling units, consisting of different cell types: osteoclasts, osteoblasts and osteocytes (fully differentiated osteoblasts embedded in the mineralized matrix and actual orchestrator of remodeling process).

Bone remodeling is highly regulated by genetic, mechanical, hormonal and local factors which determine the outcome of bone balance.

Peak bone mass is reached during puberty in women and somewhat later in males. The latter group develops a higher bone mass and present larger, wider bones, while the female bone structure tend to be smaller in diameter and cortical thickness. From about 30 years of age, a negative bone balance is observed in both sexes (with a predominance of bone resorption) which leads to a gradual loss of bone mass similar in both sexes, initially in the trabecular bone and later in the cortical.

This decline is accelerated after menopause in women due to loss of estrogens, agents with proven antioxidant properties, which maintain lower bone mass than in the case of men during aging. With age, metabolic disorders that affect the bones occur: neuromuscular changes related to lack of mobility; increased endogenous glucocorticoid production and renal failure with decreased synthesis of calcitriol. Moreover, with aging, bone collagen fibers undergo structural changes and the bone loses the ability to repair microfractures.

Most current concepts on the development of senile osteoporosis have been obtained from studies in experimental models, mainly in rodents. However, when interpreting these results, some bone peculiarities in rodents compared to humans must be taken into account, such as continuous modeling bone from the growth plate, the absence of menopause, as well as a lack of Haversian cortical bone system. However, as in humans, rodents have shown bone mass loss and a deterioration of structure and of long bone regenerative capacity associated with aging. The bone loss in aged rats is related to a decrease in osteoblast maturation and the increased number of osteoclasts compared to osteoblasts in the trabecular bone. Also, in inbred mice in which bone mass is regulated primarily by genetic factors, bone loss associated with age may assume up to 10% of the total bone mass, which is attributed to decreased bone remodeling.

As observed in rodents, humans initially tend to lose trabecular bone with age, especially in women, related in part to a decrease in physical activity and, therefore, the mechanical stimuli in the tissue. From 70 years, decreased cortical thickness is more pronounced with a concomitant increase in the intra-cortical porosity of the femur. The medullar area increases both in men and women. These changes are associated with increased risk of osteoporotic fractures. However, in both mice and humans, the mechanical properties of bone are relatively conserved through a sustained increase in sub-periosteal mineral, which increases inertia time.

Mechanisms associated with bone aging
The underlying molecular mechanisms of involutional osteoporosis have begun to be elucidated in recent years. Associated with age, there has been a decrease in the osteoprotegerin (OPG) ratio/ligand receptor activator of nuclear factor (NF). This ratio is an important modulator of the remodeled bone. Both OPG and RANKL are produced and secreted into the extracellular medium by osteoblastic cells and osteocytes. In fact, studies in mice models indicate that osteocyes produce most RANKL, thus directly influencing bone remodeling. OPG is a soluble decoy receptor that captures RANKL in the extracellular medium (or on the surface of osteoblasts) and prevents it from binding to its receptor (RANK) in cells of osteoclastic lineage, thereby preventing the maturation and activation of osteoclasts. Thus, the OPG/RANKL relationship is an important anabolic/catabolic balance factor during bone remodeling.

Several factors may affect the rate of fracture repair with age. With aging, there is a decrease in bone marrow osteoprogenitor, which occurs in parallel with increased adipogenesis. Both osteoblasts and adipocytes share a mesenchymal precursor cell differentiable either lineage depending on the microenvironment which are exposed these cells. Furthermore, osteoblasts from old...
mice RANKL production increase parallel to the decrease in expression of OPG. This alteration results in increased osteoclastogenesis and osteoclast activity^{25,26}. It is noteworthy that there are a decreased number of endothelial cells and angiogenesis, which may contribute negatively to the process of bone repair in older people^{27}. Recently an increase in bone mass and reduced risk of fractures have been observed in elderly subjects who undergo angiotensin II receptor antagonist treatment^{28}. The drug’s apparent beneficial effect on the bone is attributed to the inhibitory action of angiotensin II on various osteoblast differentiation markers, such as runt-related transcription factor 2 (Runx2), essential for osteoblast differentiation, osteocalcin^{29} and the increase of RANKL, which favors osteoclast differentiation^{30}. These data suggest that high blood pressure which is prevalent in the elderly could also contribute to involutional osteoporosis.

Sclerostin, the osteocyte-derived product of the Sost gene, is a potent inhibitor of bone formation through the binding to receptors associated with low density lipoprotein 5 and 6, inhibiting the canonical Wnt. Recent studies have shown that circulating sclerostin increases in post-menopausal women and with age in both sexes, which could have a negative influence on bone mass^{31,32}. Currently, the product of the klotho gene is known to be an important modulator of cellular aging^{33}, a transmembrane protein acting as fibroblast growth factor (FGF) co-receptor 23 produced by the osteocytes and inducer of phosphaturia. Mice deficient in the Klotho gene suffer accelerated aging and osteopenia characterized by a decrease (20-40%) of cortical thickness in the femur, tibia and vertebrae, and low bone remodeling with a very sharp decline in cortical bone formation. Stromal cells from the bone marrow of these mice have a reduced ability to mineralize nodule formation and phosphatase alkaline activity^{34}. Paradoxically, these Klotho deficient mice have increased trabecular bone in the vertebrae and the metaphysis of long bones; an effect which the authors attribute to a selective activation of the Wnt pathway on the trabecular component. Klotho interacts with the Wnt pathway through its secreted product, which binds to ligands of this pathway by inhibiting its action, hence the absence of Klotho could lead to activation of the pathway Wnt^{35}. Furthermore, mice without telomerase have been shown to exhibit increased cellular senescence and a decrease in bone mass 3 months from birth, associated with a reduction in bone formation and osteoblastogenesis^{36}. Apparently, this reduction is because mice without telomerase have poorly differentiated osteoblasts and the pro-inflammatory environment that promotes osteoclast activity.

**Oxidative stress as a pathogenic factor in involutional osteoporosis**

Aging can be seen as a consequence of the imbalance between oxidizing agents produced naturally in cell metabolism and antioxidant defenses, with a predominance of the first. This is known as oxidative stress, which involves the oxidation of biomolecules and functional loss of cells^{37,38}. Increased oxidative stress, carried out primarily in the mitochondria, is based on the overproduction of reactive oxygen species (ROS) such as superoxide anion (O_{2}^{-}), hydroxyl radicals (OH) and hydrogen peroxide (H_{2}O_{2}). This increase cannot be properly balanced by antioxidants systems such as superoxide dismutase (SOD), catalase (CAT) enzymes glutathione cycle (glutathione reductase and glutathione peroxidase) and thioredoxin, among others. Excess ROS with chronological (and/or biological) age oxidizes DNA, proteins and lipids and induces the phosphorylation of mitochondria p66^{39} protein, leading to cell death^{40,41} (Figure 1). Recently, oxidative stress has been found to have important functions in cell signaling^{42,43}. In this context, ROS can be considered second messengers of inflammatory response. In fact, oxidation and inflammation are two closely related processes that increase with age^{44}.

Although some researchers have raised questions about whether oxidative stress is a cause or consequence of aging, in recent years it has been implicated in the bone deterioration^{45}. Using various animal models: premature aging, osteoporosis due to estrogen deficit (after ovariectomy) or diabetes, increased oxidative stress markers was found to decrease bone formation mechanisms^{46,47}. The effects of oxidative stress to induce deleterious effects on bone tissue are not yet well known. Increased ROS may stabilize forkhead box O (FoxO) transcription, an important family of transcription regulators of many genes. Its functions include control of glucose metabolism, tumorigene-
sis and cell defense against oxidative stress^{48}. FoxO 1 and 3 are expressed in the bone 56, where they seem to play a key role in maintaining bone formation^{49}. It has been shown that genetic deletion of FoxOs in mice increases oxidative stress in bone and induces bone loss trabecular and cortical, associated with increased osteoblast/osteocytic apoptosis and a decrease bone formation^{50}. The activation involves FoxO phosphorylation engagement with the beta-catenin^{49} causing gene induction of oxidative stress response, as GADD45 and CAT^{51}. In fact, the protective action of oxidative stress of Klotho protein appears aforementioned mediated activation FoxOs^{52}. Furthermore, activation of the FoxO prevents beta-catenin to act as transcription factor in stimulating the proliferation and differentiation of osteoblasts^{53}.

Increased ROS in bone cells causes damage and apoptosis genomic DNA of osteoblasts and osteocytes. In addition, lipid peroxidation dependent lipoxygenase activated by oxidative stress plays an important role in bone loss associated with aging. This is evidenced by analyzing the expression of the lipoxygenase and ALOX12 and formation Alox15 4-hydroxynonenal, a product of lipid peroxidation, increased bone in older mice^{54}. It has also been shown that products of lipid oxidation inhibiting action osteogenic factors^{55}.
Furthermore, the increase of ROS has been linked to an increase of osteoclastogenesis and osteoclast activity. It has recently been shown that the enzyme nicotinamide adenine dinucleotide phosphate oxidase 4 (NOX 4) plays a key role in osteoclastogenesis. Mice deficient of this enzyme, which produces constitutively ROS have a high bone mass and osteoclast markers deficit; also in human bone samples high osteoclast activity is correlated with increased activity of NOX 4. Furthermore, it is noted that in situations of increased ROS associated with experimental DM, are mixed results. While some authors have observed an increase in osteoclast activity, it has been suggested that could be related to the greater severity of DM, however, other DM models, osteoclastic activity is reduced. In fact, studies using murine osteoclasts pre-incubated in the presence of high glucose appear to confirm their inhibitory effect on osteoclasts. Thus, differences in the degree of DM, strain and age of the animal, could contribute to the varying levels of bone resorption observed in different models.

**Possible oxidative stress therapies in senile osteoporosis**

The development of new anabolic therapies for osteoporosis that combine increased bone mass with its ability to neutralize the harmful effects of oxidative stress is of great interest. An intuitive approach to prevent bone loss with age would be based on the antioxidant administration. However, it pointed out that classic antioxidants, such as the CAT or N-acetylcysteine, exert undesirable effects on bone tissue as authentic anti-osteoclastogenic act as agents interfering with bone remodeling. In addition, such agents inhibit the canonical Wnt/beta-catenin whose activation is vitally important for maintaining bone formation, partly by inducing the seizure of activating the protein disheveled by the regulatory protein redox balance, nucleoredoxin.

Recently, the bone anabolic effect has been associated with intermittent administration of parathyroid hormone (PTH) with its stress oxidative properties, such as the decrease in the amount of ROS, inhibition of phosphorylation of p66shc adaptor protein and increasing the amount of total glutathione. The advantage of this treatment with PTH versus the clas-

Figure 2. Osteogenic actions of PTH through the Wnt/beta-catenin pathway. PTH is able to directly activate the Wnt pathway by binding of the type 1 receptor (PTH1R) with coreceptor Wnt proteins, the related receptor low-density lipoprotein 6 (LRP6). Furthermore, phosphorylation of Akt activation produced by PTH1R results in FoxO degradation, which favors beta-catenin stabilization.

Conclusions

The progressive aging of the population in the developed world leads to increased musculoskeletal disorders, including osteoporosis. Osteoporosis and increased fragility of the elderly population are a socioeconomic challenge of the first magnitude. Different factors contribute to bone loss in the elderly, among which stands out as a common element increased oxidative stress (Figure 4). Thus, reducing oxidative stress could be a useful tool to combat involutional osteoporosis. However, the fact that oxidative stress compounds could interfere with the bone remodeling or key anabolic pathways for bone formation, such as the Wnt signaling pathway, requires certain considerations prior to therapeutic use. We must also take into account the physiological role of ROS, which act as secondary messengers of many metabolic pathways; therefore its uncontrolled inhibition could lead to unwanted side effects in bone cells. Further research is needed to determine the true effect of antioxidant therapies and appropriate dosing schedules to avoid deleterious action on bone remodeling. Taking into account these considerations, therapies aimed at neutralizing oxidative stress to prevent or alter the course of involutional osteoporosis would represent an obvious medical breakthrough.

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Bibliography


Figure 4. Role of oxidative stress in bone damage associated with aging. Alteration of mitochondrial homeostasis with age causes generation of excessive amounts of ROS that exceed the capacity of cellular detoxification systems. Bone-forming cells in, excess ROS results: an increase in receptor expression activated gamma peroxisome proliferator (PPAR-gamma3); FoxO coupling with PPAR beta-catenin, which inhibits the Wnt pathway; and p66 protein phosphorylation inducing apoptosis. This excess ROS favors increased osteoclast activity and osteoclastogenesis. Together these facts altered bone remodeling. The oxidative stress activity of agents such as PTH (and PTHrP), resveratrol and salubrinal are associated with osteogenic actions.

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