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Reactive Oxygen Species and Bone Fragility

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Abstract

Reactive oxygen species (ROS) are key signaling molecules that play an important role in the progression of inflammatory disorders. In the last decade, studies have indicated that ROS, including superoxide and hydrogen peroxide, are crucial components that regulate the differentiation process of osteoclasts. Osteoclasts (OCs), cells specialized for bone resorption, utilize ROS as second messengers during receptor activator of NF- κ B ligand (RANKL)-induced differentiation and activation. The purpose of this chapter is to explore the current understanding of reactive oxygen species involvement in bone pathophysiology.

Keywords: fragility fractures, free radicals, bone, osteoporosis, oxidative stress

1. Introduction

Reactive oxygen species (ROS) are known to determine oxide-reducing balance alteration, oxidative stress, and carcinogenicity. Many diseases, including cancer and other pathologies associated, like arteriosclerosis and cataracts, are related to mitochondrial dysfunctions provoked by reactive oxygen species [1, 2]. Free radicals ($O_2^{\cdot-}$, HO^{\cdot} , H_2O_2) react easily and cause damage to the DNA and cell membranes, generating strong oxidant agents inside cells [3]. Reactive oxygen species (ROS) play a role in a number of degenerative conditions including osteoporosis [4].

Bone is an important organ performing three essential physiological functions: mechanical support, mineral homeostasis, and support of hematopoiesis. Bone diseases in the elderly are associated with increased morbidity and mortality. Osteoporosis is one of the most important diseases that affects the quality of life for the elderly [5]. Although the bones stop growing after adolescence, the bone is a very dynamic tissue. Bone tissue is continuously reabsorbed and regenerated by constantly changing its structure.

One of the most striking features of the bones is their ability to reshape. This remodeling process occurs during growth and continues throughout its life. During bone formation, the bone is formed and deposited, according to a particular pattern, by a process called ossification. The remodeling is continuous, leading to the organization of the bone structure in regular units, allowing the bone mass to gain maximum resistance to the mechanical forces acting on it. The older bone is removed by osteoclasts (bone-resorbing cells), and osteoblasts (bone-forming cells) lead to the deposition of new bone tissue. Osteoclasts secrete enzymes that break down the bone matrix as effectively as acids and convert calcium salts resulting in a soluble form (that can be absorbed into the bloodstream).

Osteoclast activity occurs behind the epiphyseal growth zone to reduce bone margins enlarged at the width of the bone stem that undergoes an elongation process.

The remodeling process is especially important for long bones supporting the limbs. These bones are widest at the ends and narrower in the middle, which gives added strength to the joints.

As osteoclasts destroy the old bone at the epithelial ends of the bone, the osteoblasts within the growth zone create a new epiphysis.

In each of the tubular spaces released by osteoclasts within the bone, the osteoblasts come in to deposit a new bone layer.

A bone that is poorly used, such as a lower immobilized member after a trauma, will be prone to reabsorption because bone destruction goes beyond bone formation.

Bones subjected to increased stress are permanently remodeled. The femur, for example, is completely replaced every 6 months.

Bone remodeling gives the different form of long bones. They are wider at each end and thinner in the middle. Bone remodeling not only changes the bone structure but also helps regulate the level of ionic calcium in the blood. Calcium is necessary for normal nervous transmission, for cell membrane formation, and for blood clotting. The resorption and bone formation sequence is shown in **Figure 1**.

Osteoblasts (bone-forming cells), osteoclasts (bone-resorbing cells), and osteocytes (load-sensing cells) are the cell types participating in bone remodeling. During remodeling, these cell types are spatially and temporally organized in functional structures called basic multicellular units (BMUs). The remodeling sequence operated by a BMU follows well-defined phases [5]. The bone remodeling sequence is influenced by a number of regulatory factors produced by hormonal glands, bone cells involved in sensing the mechanical environment, lymphocytes, and even tumor cells [6].

Osteoporosis is characterized by the parallel reduction of bone minerals and bone matrix so that the bone is in a low amount but with a normal percentage composition.

Despite rapid advances in our understanding over the last few years, the morbidity and mortality of patients resulting from this disease are still too high [4], and there is an urgent need for a proper assessment of the underlying mechanisms and the development of new treatment strategies to address this pathophysiological issue.

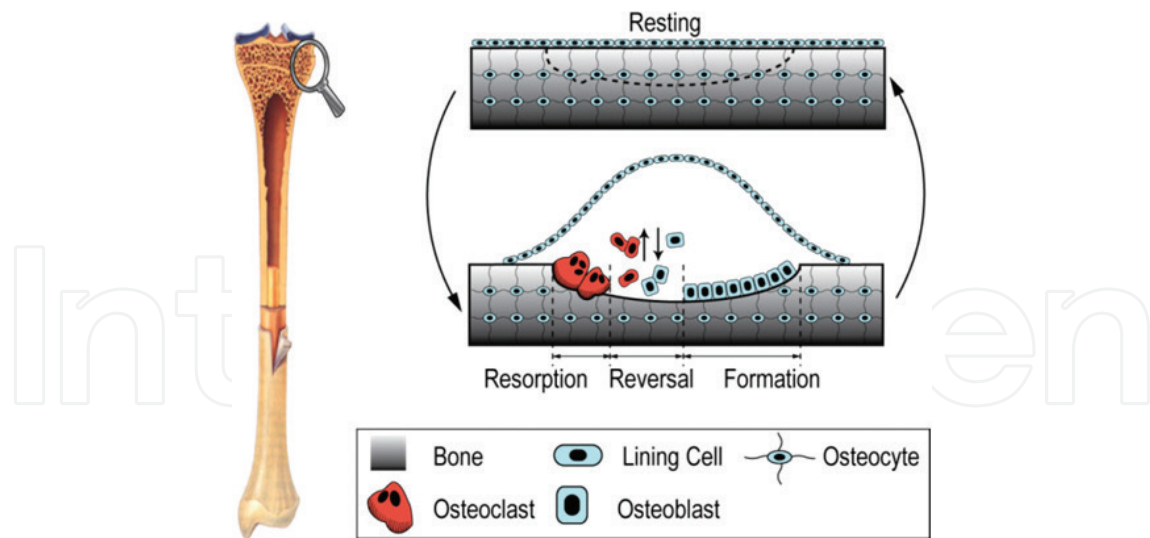


Figure 1. Bone remodeling sequence executed by different bone cell types within basic multicellular units (BMUs) (figure from Ref. [5], reproduced with permission).

2. Reactive oxygen species

Oxygen (O_2), nitrogen oxide (NO), and iron (Fe^{2+}) are present in erythrocytes. These elements may in some conditions form reactive species, called radicals that affect red blood cells and vascular endothelium. A chemical species that presents an unpaired electron in outer orbitals and which can exist independently is called free radical. Radicals are very reactive trying to complete their own orbits by taking an electron from a neighboring molecule. Oxygen and nitrogen are the two chemical elements that in the body give rise to reactive species called the reactive oxygen species (ROS) and reactive nitrogen species (RNS), respectively.

2.1. ROS: general definitions

Reactive species can be defined as atoms or molecules that contain in their structure an odd electron that causes an atom/molecule energy instability, which is reflected in their increased reactivity.

Reactive species are produced in the body for physiological purposes in defense, vaso-relaxation, cellular or accidental exposure to toxic, or radiation exposure. When the amount of reactive species exceeds nontoxic physiological levels, the so-called oxidative stress occurs. Oxidative stress can be defined as the status of reactive oxygen or oxygen radicals in a biological system. It results from the imbalance between oxidants and antioxidants, in favor of oxidants, with destructive and pathogenic potential [7, 8].

The reactive species of oxygen are superoxide ($O_2^{\bullet-}$), hydrogen peroxide (H_2O_2), hydroxyl radical (OH^{\bullet}), and singlet oxygen (1O_2). Free oxygen radicals (ROS) produce harmful effects on the body called oxidative stress (OxS). The reactive oxygen species are constantly formed as reaction products in all cells by aerobic processes such as oxidative phosphorylation.

The base state of oxygen is triplet. This means that it has two unpaired electrons that are positioned on two different molecular orbitals.

Because electrons have parallel spin, oxygen can only rotate in the same orbit. Since molecular oxygen is paramagnetic, it is concluded that it has the structure represented in the right-hand side of **Figure 2**. Molecular oxygen can react rapidly with single unpaired electrons of transitional metals (e.g., Fe, Cu, Mn). One mole of properly chelated copper could catalyze consumption of the oxygen in an average room within 1 s in [9]. Depending on the conditions, O_2 can be both kinetically stable (not reactive) and very reactive.

For proteins and enzymes that have a metal in their structure, the oxidative attack of O_2 is slow, and in conclusion the superoxide radical will slowly form.

Superoxide (O_2^-): this radical constitutes the first step of oxygen activation by way of univalent reduction, forming in most of the autoxidation reactions by the capture of an electron that is placed on one of the antiknocking orbital n (**Figure 3**).

After an electron has been acquired, the following electrons are added more easily, and the reactions take place faster, leading to the generation of reactive oxygen species [10].

Reducing the hydroxyl radical to water takes place in the last stage of electron acceptance (**Figure 4**).

Superoxide radical (O_2^-) can react in the presence of ferric iron with hydrogen peroxide generating the most potent hydroxyl radical through a nonenzymatic reaction named Haber-Weiss reaction.

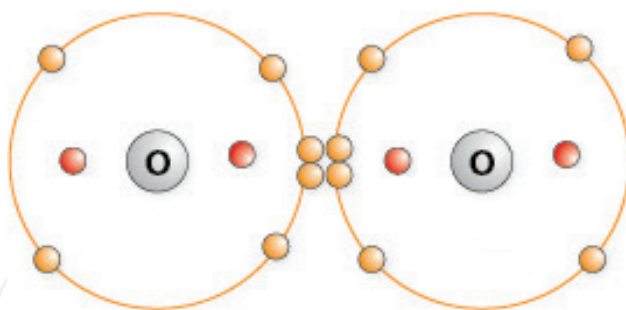


Figure 2. The structure of oxygen molecule.

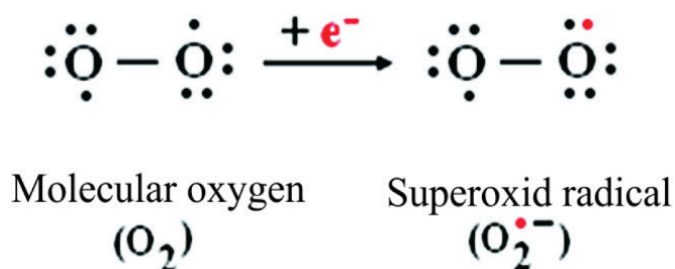


Figure 3. Superoxide radical generation.

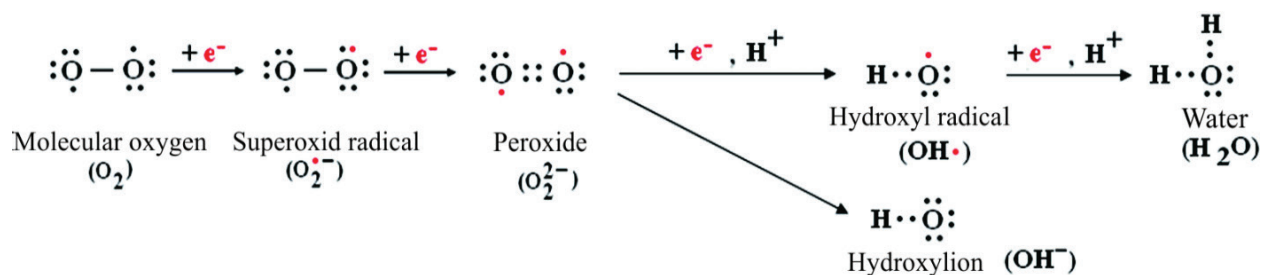


Figure 4. Reactive oxygen species generation.

The reaction involves two steps:



The nonenzymatic reaction occurring in living cells is:



In the presence of ferric iron, superoxide radical is a source of hydroxyl radical.

Most of the superoxide radicals are formed by electrons derived from the coenzyme Q cycle in complex III and by NADH flavoprotein dehydrogenase (complex I). The superoxide radical, which has a relatively low degree of toxicity compared to other reactive oxygen species, is kept undetected by enzymes called superoxide dismutase (SOD) that catalyzes the dismutation reaction.

Hydrogen peroxide (H_2O_2) is not a free radical due to the fact that it has no unpaired electron in the structure, which gives it reactive stability, being the most easily measurable reactive species. H_2O_2 is a weak oxidizing agent that forms as a result of the O_2 -dismutation reactions or oxidase-catalyzed reactions. This process takes place at the level of peroxisomes, cellular organisms with rich enzyme equipment. Concentration of hydrogen peroxide is controlled by catalase (present in peroxisomes) and by glutathione peroxidase (localized to the cytosol).

Hydroxyl radical (OH^{\cdot}) is a highly reactive free radical which diffuses over small distances until it reacts with any organic molecule encountered such as carbohydrates, amino acids, lipids, nucleic acids, and organic acids. The toxicity of this molecule comes from the fact that it can initiate chain reaction.

Singlet oxygen ($^1\text{O}_2$) can result from oxidoreduction reactions. Single oxygen is more reactive than the hydroxyl radical by reacting with many organic compounds such as polyunsaturated fatty acids, cholesterol-forming hydroperoxides.

Singlet oxygen can be formed from superoxide radical in [11]:



Although it is not a radical, singlet oxygen is more reactive than the hydroxyl radical. The most reactive radical is hydroxyl (OH^\bullet) which takes electrons from other molecules around it, whereas superoxide (O_2^\bullet) and hydrogen peroxide (H_2O_2) are more selective in their reactions with various molecules [12].

The processes so far presented above take place in all human cells. The molecular oxygen that is transported to the tissues is found for a short period of time unbound. It is assumed that in this state, it generates the radicals previously presented. Oxygen binds to hemoglobin molecule at the ferrous iron (Fe^{2+}). Some of Fe^{2+} is transformed by O_2 to ferric form (Fe^{3+}) in resulting methemoglobin. Methemoglobin reductase is an enzymatic system which restores Fe^{3+} to Fe^{2+} and reduces methemoglobin back to hemoglobin. Oxygenated hem has some of the electronic characteristics of a $\text{Fe}^{3+}\text{OO}^-$ peroxide anion [10]. Like in this study [13], the author found that the $\text{Fe}^{3+}\text{O}^{2-}$ complex is able to generate superoxide radical during the normal molecular oxygen transport to tissues through the hemoglobin auto-oxidation.

Some researchers propose that hemoglobin may have oxidative reaction in the oxygen-releasing process.

Like in this study from 2006 [14], the author demonstrates that at intermediate oxygen pressure, where hemoglobin partially releases molecular oxygen, the superoxide radical production increases. They show that superoxide radical is released in the hydrophobic hem pocket.

Through the Haber-Weiss reaction, H_2O_2 , even if it is not a radical, can form short and very active hydroxyl radicals [15]. The hydroxyl radical (OH^\bullet), which is highly reactive, reacts with any biomolecules it encounters. There are studies according to which the high reactivity of the hydroxyl radical decreases its ability to diffuse [16]. Since the hydroxyl radical may generate an autocatalytic reaction in the chain, we can say that it is particularly dangerous.

There are some researchers who believe that the hydroxyl radical is responsible for cellular damage and others that ferric ion promotes initiation of the chain reaction [17–19]. In conclusion, both oxidative species can form in living cells [20].

Like in **Figure 4**, ROS are produced accidentally and physiologically in various enzyme-catalyzed reactions.

2.2. Effects and signaling of ROS

Reactive oxygen species can interact with any biological molecule causing damage to lipids, proteins, and DNA. Prospects for effective therapeutic intervention may fail if the initial target of oxidative stress is unknown. For example, it is known that DNA is the primary target of lesions produced by the addition of H_2O_2 to mammalian cell cultures, so that DNA strand breaks occur before lipid peroxides or “detectable” oxidized proteins. The method of detecting lesions produced on target molecules may give incomplete information. The evidence of protein damage by detecting carbonyl radicals in the initial stages of lesions may be negative, but the determination of SH oxidation, which occurs earlier, is positive. During peroxidation

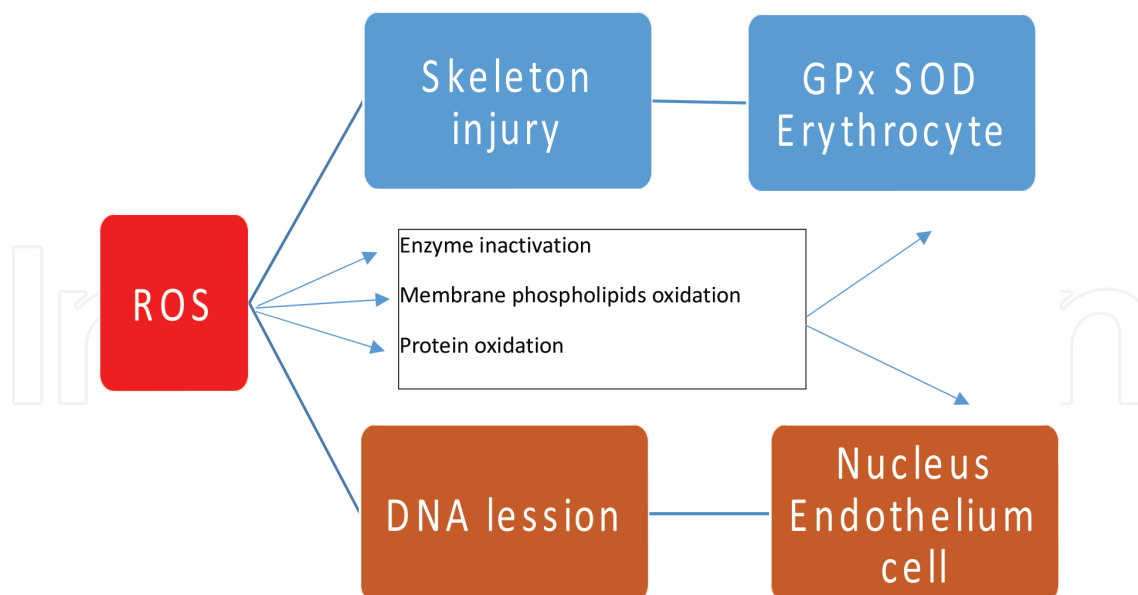


Figure 5. ROS damaging activity.

of lipids, the peroxy radicals are formed by chain reactions. These intermediates that are formed can amplify the lesion. The attack of ROS affects both erythrocytes and endothelial cells. DNA damage may occur in nucleus containing endothelium cell besides lipid and protein oxidation. Interaction between free radicals and DNA can lead to strand breaks or structural changes such as adduct formation (**Figure 5**) [21, 22].

In general, lipid peroxidation occurs in late stages of aggression, so therapies directed against lipoperoxidation may be less beneficial. This is another reason that can lead to erroneous conclusions about the importance of oxidative stress and antioxidant therapies.

As long as they do not accumulate in excess, ROS have positive effects in the body, being involved in various cellular mechanisms. For example, biosynthesis of thyroid hormones involves the formation of hydrogen peroxide in order to assemble thyroglobulin iodine to synthesize thyroxine. On the other hand, in the immune response, in neutrophils and macrophages, NADPH oxidase catalyzes the formation of superoxide anion by internalizing

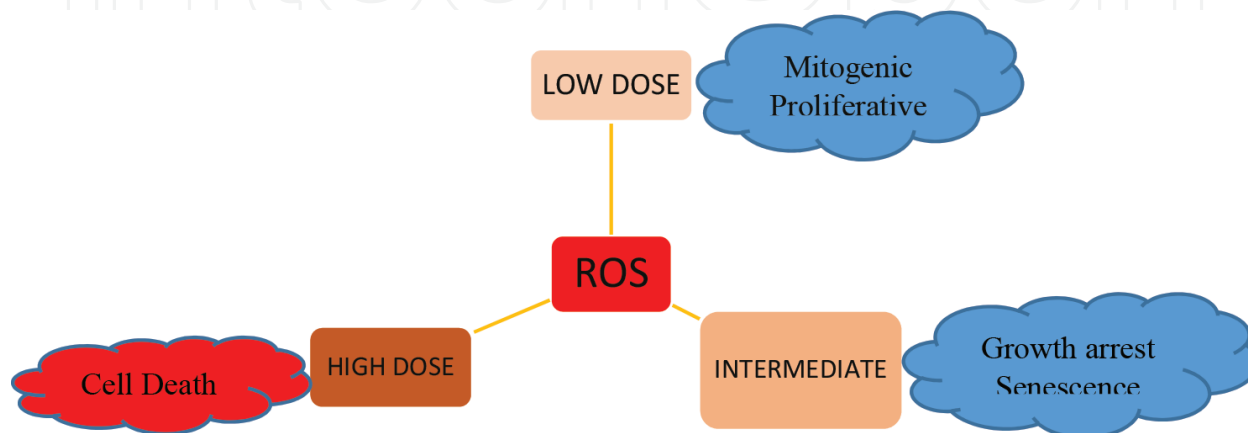


Figure 6. Cells response under ROS attack [23].

bacterial antigenic structures. The process is completed by the action of SOD, which will produce hydrogen peroxide that will induce bacterial lysis. It has been shown that ROS acts as a signaling molecule [21, 22]. In response to the intensity of attack of the ROS, cellular responses are triggered, which prepare the cells to survive or to die (**Figure 6**).

Oxidative stress can trigger cellular response through different signaling modes. ROS modulates different types of enzymes, the activity of transcription factors, and the ionic channel. Both kinases and phosphatases can be modulated by ROS.

Mitogen-activated protein kinase (MAPK), which has three subfamilies, namely, N-terminal c-Jun kinase (JNK), p38 MAPK, and extracellular signal-regulated kinase (ERK), is part of the kinase class [24]. These MAPK pathways are structurally the same. The difference between them is found at a functional level. ROS activates these three MAPK pathways [25] and may inhibit tyrosine phosphatase activity.

In gene and protein expression, ROS are involved by activating transcription factors.

The plasma membrane Ca^{2+} and K^{+} channels are stimulated by ROS. The increase in calcium ion concentration ROS mediated leads to the oxidative stress-mediated activation of PKC and to the transcriptional induction of the AP-1 proteins c-Fos and c-Jun [26].

Cells can be protected from attack by reactive species through various mechanisms. Antioxidants can be divided into two groups: enzymatic like superoxide dismutase (SOD), glutathione peroxidase (GPx), and catalase and nonenzymatic like vitamins E and C, provitamin A (b-carotene), and glutathione.

SOD is found in all aerobic cells as well as in aerobic optional bacteria. Initially, it was interpreted as a copper storage form, and later three types of SODs were described, based on the type of metal in the catalyst center.

The role of superoxide dismutase is to catalyze the dismutation reaction of the superoxide radical anion according to the equation (**Figure 7**):

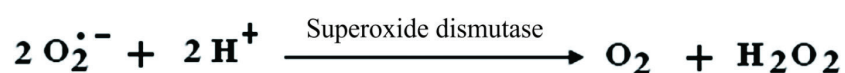


Figure 7. Superoxide dismutase activity.

The enzyme may also inhibit the production of singlet oxygen and indirectly the peroxidation of polyunsaturated fatty acids. In addition to the role of the oxidative marker, SOD is tested as a potential therapeutic agent under pathological conditions in which the oxidative stress has a clear role, such as ischemia-reperfusion syndromes, hepatic disorders and acute and chronic inflammation, cataracts, rheumatic arthritis, diabetes, and neoplasia. One of the enzyme-catalyzed reactions leads to the appearance of another harmful compound—hydrogen peroxide [27].

GPx is part of a family of enzymes catalyzing the degradation of H_2O_2 resulting from normal metabolic processes and providing the protection of proteins, lipids, and nucleic acids from the action of oxidizing molecules, using glutathione as an electron donor or, in some cases, thioredoxin or glutaredoxin (**Figure 8**).

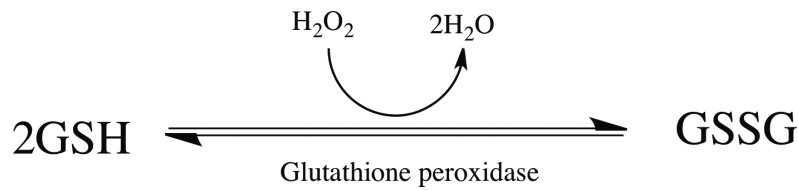


Figure 8. Glutathione peroxidase activity.

GPx is a dependent selenium enzyme, found in cytosol (70%) and in mitochondria (30%). It is irreplaceable in the antioxidant arsenal, especially in mitochondria, as they do not contain catalase for peroxide metabolism. GPx also provides protection against organic H₂O₂ and helps to regenerate the reduced vitamin C form. Imbalances in the GPx level have been observed with aging and a variety of disorders such as cancer, cardiovascular disease, and diabetes [28].

GPx is an enzyme that has a potentially greater antioxidant than SOD and catalase due to the wide-specific substrate. Other roles of GPx are regulating prostaglandin biosynthesis by inhibiting lipoxygenase and, in conjunction with glutathione reductase, contributing to the restoration of reduced glutathione.

3. Oxidative stress in bone remodeling

Bone tissue undergoes, throughout life, a continuous renewal through a process called bone remodeling, which is controlled by the activity of osteoclasts mediating bone resorption and parallel activity of osteoblasts which mediate bone formation [29]. A bone remodeling cycle involves three stages: (1) initiation, during which osteoclasts are formed and resorb damaged bone; (2) reversal, the transition of osteoclast to osteoblast activity; and (3) formation, when osteoblasts replace the portion of the bone that was resorbed [30]. The hormonal imbalance or the aging process can lead to disruption of the balanced formation process and bone resorption. This may result in decreased bone mass and osteoporosis, which increases the risk of fractures. A very important factor underlying the pathogenesis of osteoporosis is the receptor activator of NF-κB ligand (RANKL). It plays an important role in osteoclast differentiation and activation [31]. For this reason, inhibition of RANKL represents an innovative therapeutic target for controlling osteoclastogenesis [32].

In the bone remodeling process, the alternative NF-κB pathway, which mediates the activation of the p52/RelB NF-κB complex, is involved. The difference of the alternative mechanism is represented by p100 processing of a NF-κB2 precursor protein. Both NF-κB-inducing kinase (NIK) and IKKα, a downstream kinase, promote induction of phosphorylation-dependent ubiquitination for p100. NF-κB-inducing kinase is processed by a tumor necrosis factor (TNF) receptor-associated factor-3 (TRAF3)-dependent E3 ubiquitin ligase. NIK activates the alternative path of NF-κB. This occurs after signals mediated by a subset of TNF receptor superfamily members [33]. The inhibitory role of p100, in both basal and stimulated osteoclastogenesis in bone formation and resorption, has been clearly demonstrated [34]. In the alternative NF-κB pathway p52 derived from p100 through NIK, binding of p52 and RelB induces effects on osteoclast biology [34]. However, to date, the precise physiologic importance of

alternative NF- κ B in bone biology is not completely elucidated. Furthermore, the currently known intracellular signaling pathways activated after receptor binding of RANKL include the nuclear factor of activated T cells [35], mitogen-activated protein kinases (MAPKs), TRAFs, c-Jun N-terminal kinases (JNKs), and ROS [36, 37]. In addition, NF- κ B is a transcription factor, which pleiotropically regulates osteoclast formation, function, and survival [35].

The reactive oxygen species are involved in the regulation of RANKL-dependent osteoclast differentiation. They act as intracellular signaling compounds. ROS have cytotoxic effects, including lipid peroxidation and DNA damage.

Since the relationship between Nrf2 and osteoclastogenesis is known, stimulation of precursors of osteoclasts with RANKL will lead to the upregulation of Keap1 and downregulation of cytoprotective enzymes. Osteoclast precursors are primary peritoneal macrophages and RAW 264.7 cells. Keap 1 is a negative regulator of Nrf2. However, overexpression of Nrf2 leads to the regulation of the expression of heme oxygenase-1 and gamma-glutamylcysteine synthetase enzymes associated with decreased reactive oxygen species and slowing of bone destruction [38]. Consistent with this line of evidence, overexpression of Keap1 or RNAi-induced knockdown of Nrf2 resulted in effects opposite to those obtained by stimulation of Nrf2-dependent DNA binding activity [38]. We still do not know the exact mechanisms by which RANKL stimulation reduces Nrf2. It is known that Keap1 has in its structure thiol groups. These groups are highly reactive, and oxidation at their level produces conformational changes to Keap1. Ultimately these will lead to dissociation from Nrf2 and promotion of nuclear Nrf2-dependent DNA binding activity [38].

In addition, Nrf2 autoregulates its own expression [39–41]. Taken together, this evidence implies that an increase in ROS levels induced by stimulation with RANKL may upregulate Nrf2. It has also been reported that Nrf2 regulates Keap1 by controlling its transcription [39–41].

Decreasing translation through miRNA can modulate downregulation of Nrf2. The downregulation of Nrf2 may be modulated by decreasing translation by miARN or by modifying the mRNA stability Nrf2. Bach1 is an inhibitor of Nrf2 binding to ARE. This inhibitor is implicated in the mechanism indicated by the inhibited osteoclastogenesis discovered in Bach1 knockout mice [38]. In conclusion, the Keap1/Nrf2 axis regulates RANKL-dependent osteoclastogenesis both by modulating intracellular ROS signals and by expressing cytoprotective enzymes. Elucidation of the precise mechanism linking Nrf2 to stimulation with RANKL may be a therapeutic treatment for destructive bone disorders through the Keap1-Nrf2 axis [42].

4. Oxidative stress in the establishment of bone disease

4.1. Osteoporosis

The human aging implies a failure in bone formation and a loss of bone mass, but the specific molecular mechanisms arbitrating these effects are still unclear. Numerous studies in experimental animals have offered arguments for a damaging effect of oxidative stress in bone tissue, supporting the idea that an increase in reactive oxygen species (ROS) with advancing age characterizes a pathophysiological mechanism essential in age-related bone damage. The disproportion between

bone resorption and formation with age is related to various factors. A reduced wall width represents the most common histological feature in aged human bone due to a reduced deposition of bone matrix related to an insufficient number of osteoblasts involved in bone remodeling [43, 46]. It was found that the low number of osteoblasts in the aging bone is correlated with a decrease in the number of mesenchymal stem cells and improper proliferation and differentiation of progenitor cells [45]. An additional histologic feature of aged human bone tissue is a decrease in osteocyte density in the structure of bone lamellae accompanied by a mineralization of osteocyte lacunae. The precise mechanism of this phenomenon—named micropetrosis—is not very well known, but it seems to contribute to the decrease in osteocyte density with age [44–46].

The histopathological examination reveals thinned bone trabeculae that lose continuity being separated from each other by enlarged areolae with adipose degeneration of the marrow. The reduction of the trabecular connectivity is related to osteoporosis stages. The decrease of the medullar cellularity together with its enhancement in fat cells has negative outcomes on the bone. The areolar spaces are stretched, bordered by incomplete bone septa. This fact could be explained by the decrease of the connectivity of the bone trabeculae that will lead to the connection of the areolae by the osteolysis of certain walls (**Figures 9 and 10**).

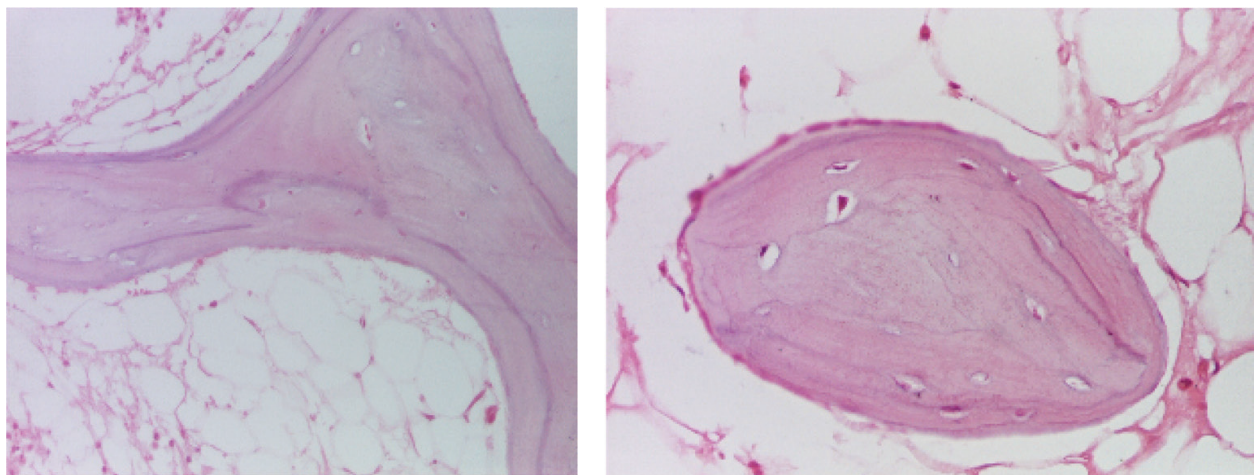


Figure 9. Osteoporotic bone—hematoxylin-eosin (HE) stain.

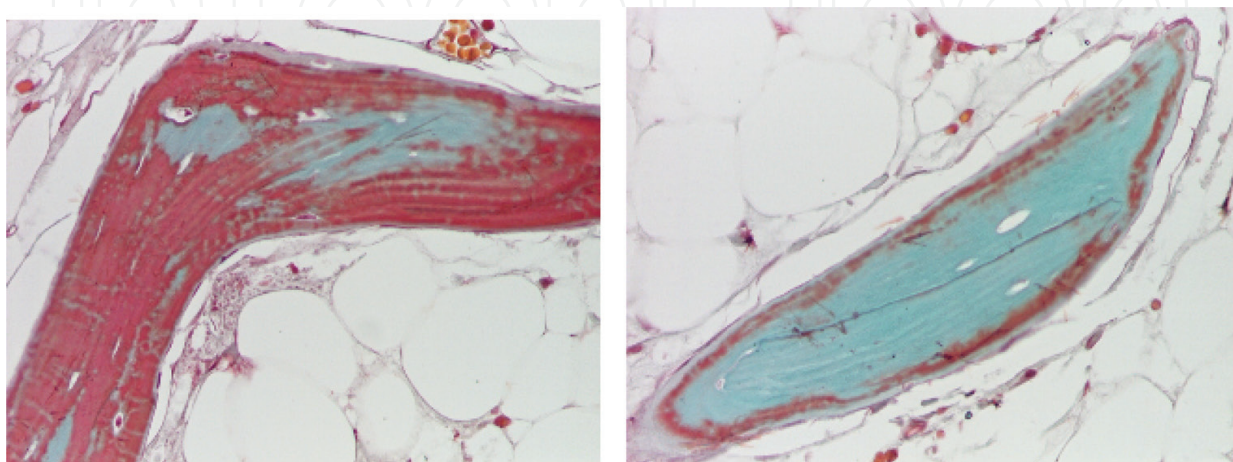


Figure 10. Osteoporotic bone—trichromic Goldner-Szekely stain.

4.2. Bone tumor development

Healthy people have a balance between bone formation and bone resorption. In various bone diseases, even in malignancy, loss of this balance leads to damage to the normal structural integrity of the skeleton [47, 48]. Tumor cells act by excessive stimulation of both osteoclasts and osteoblasts.

Oxidative stress is one of the most important events that gives rise to the conditions leading to tumor onset and progression [49]. Reactive oxygen species (ROS) is one of the most important species of free radicals. ROS controls many cellular processes, including cell proliferation, and thus stimulates the uncontrolled cell growth which may lead to tumor development [50]. In the case of chronic inflammation, the secretion of ROS may lead to the amplification of dysregulated processes and eventually to the development of a pre-neoplastic state.

When the endogenous antioxidant response is exceeded by the amount of ROS produced, oxidative damage can occur in the lipids, proteins, and DNA. These lesions can lead to genetic alterations and ultimately to dysregulation of suppressor genes. Since oxidative stress and chronic inflammation processes are linked, failure to block these processes can lead to the initiation of carcinogenesis through genetic alterations [51]. In patients who have cancer, low total antioxidant capacity can be detected even before oncological treatment is started [52].

4.3. Diabetes-associated bone complications

Diabetic patients have been shown to increase lipid peroxidation and decrease the activity of erythrocyte antioxidant enzymes. Hyperglycemia is due to impaired insulin secretion in T1DM and insulin resistance in T2DM.

In hyperglycemic conditions, oxidative stress occurs as a result of the increased activity of the polyol pathway [53], the hexosamine pathway [54–56], and the promotion of the activator of protein kinase C [57]. Hyperglycemia also leads to a higher activation of the nuclear-kappa B (NF- κ B), by protein kinase C in vitro. The nuclear factor-kappa B is a central transcription factor involved in the regulation of many proinflammatory genes, including cytokines (TNF-tumor necrosis factor, interleukin) and hematopoietic growth factors [58]. Under hyperglycemic conditions, the NADH/NAD⁺ + cytosolic ratio increases because sorbitol is oxidized by NAD⁺. In this situation, the activation of the glyceraldehyde-3-phosphate dehydrogenase enzyme is inhibited. Although there is still little research, type 1 diabetes has been associated with osteoporosis. Therefore, we can say that type 1 diabetes may increase the risk of fractures [59]. Type 1 diabetes affects both bone density and bone quality [60, 61]. In T2DM, BMD is equal or increased according a meta-analysis, but the fracture risk is increased despite this increase in BMD [62, 63].

Osteocalcin (OST) is the second structural protein after collagen, a component of bone tissue, mainly involved in the process of bone mineralization and calcium homeostasis. It is synthesized by osteoblasts, under the action of vitamins K and D₃, from a pre-pro-osteocalcin precursor made up of 98 amino acids. Clinical research has shown that serum osteocalcin levels

are significantly lower in patients with type 2 diabetes, becoming normal after improved glycemic control. Osteocalcin is an independent factor associated with glucose and glycated hemoglobin for menopausal women.

It has been revealed that diabetes causes a reduction in the number of osteoblasts [64]. Diabetes can affect bone-forming cells through increased apoptosis. Like in this study from 2007, authors found that AGEs induced apoptosis of osteoblasts through the MAP kinase pathway [65]. Another mechanism by which diabetes is associated with bone formation is the reduction in the expression of transcription factors involved in the regulation of osteoblast differentiation [66]. Studies in rats with diabetes showed a decrease in both alkaline phosphatase activity and the formation of the mineralized matrix [67, 68].

RAGE, the AGE receptor, is expressed at higher osteoblast levels in rats with diabetes, thus rendering the animals more susceptible to AGEs effects [69].

Some enzymes called serine kinases can be induced by oxidative stress. Their induction affects the ability of insulin to activate protein kinase B and glucose transport. When insulin action is impaired, diabetic complications may occur. In this context, the NF- κ B, p38 MAPK, and JNK/SAPK pathways are sensitive to oxidative stress [70].

A considerable amount of evidence has accumulated indicating that metabolic and endocrine alterations caused by diabetes affect bone quantity and quality over the last decades of life [71].

Hyperglycemia-induced oxidative stress is a major contributor in the development of long-term complications of diabetes mellitus [72–75].

5. Conclusion

In this chapter we discussed about the role of oxidative stress in bone pathophysiology and the possibility of ROS production being a relevant therapeutic target under certain conditions.

Bone remodeling is a process of continuous formation and resorption occurring in specific areas of the matrix. Novel therapeutic strategies have been developed that focused on the inhibition of excessive bone resorption and promotion of bone formation process. As in this study from 2017 [47], the author proposed as osteoblast inhibitors in dexamethasone (Dex)-induced apoptosis the synthetic derivatives of benzo[1,2,5]selenadiazole (SeDs). These compounds may have a protective effect. Dex treatment leads to overproduction of ROS in the cells, DNA fragmentation, caspase-3/caspase-9 activation, p53 phosphorylation, and MAPK-pathway activation. When cells were pretreated with SeDs, these modifications were blocked, suggesting to the authors that SeDs may present a therapeutic application to antagonize osteoporosis induced by glucocorticoids [47]. Accordingly, basic research can contribute to the identification of specific pathways that can be effectively targeted by novel compounds able to treat and possibly reverse osteoporosis, particularly which occur in already chronically severed patients, such as in neurodegenerative disorders. In conclusion, we have shown that osteoclast differentiation and bone resorption are associated with the generation of ROS and oxidative stress. These data also indicate that by reducing bone resorption, antioxidants have the potential to treat osteoporosis.

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