The role of melatonin in periodontal and periimplant bone homeostasis and regeneration

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Abstract

Background

Melatonin, a hormone produced primarily in the pineal gland, possesses a series of biological properties that appear to have an influence on bone homeostasis. Currently, little is known about how melatonin influences bone metabolism in periodontology and implantology.

Objectives

The objectives of this study are (1) to review the properties of melatonin in regulating bone homeostasis; (2) to discuss its direct and indirect effects on bone; and (3) to propose mechanisms for the use of melatonin as an agent to promote alveolar bone regeneration.

Conclusion

Melatonin positive regulation of bone formation and homeostasis, in combination with the inhibitory effects on bone resorption, highlights the potential use of melatonin as a marker of periodontal and periimplant bone-related diseases. *In vitro* and animal studies show promising results on the use of melatonin as a regenerative agent, although no clinical studies have yet been performed.

Keywords

Melatonin, osteoblasts, osteoclasts, periodontal disease, dental implant, free radicals.

Introduction

Numerous systemic hormonal changes are known to be associated with aging.[1] Some conditions linked to circadian rhythms and age may alter bone metabolism, resulting in changes in immune activity or bone-associated pathologies,[2] such as periodontal disease. These disorders may be associated with alterations in normal levels of melatonin.[3, 4]

Melatonin (*N*-acetyl-5-methoxytryptamine), a hormone that is endogenously synthesized, primarily in the pineal gland, is a molecule with intense antioxidant activity[5] and a wide range of biological actions, notably in the control of metabolism and bone development.[6] Melatonin is currently used in therapies as a coadjuvant in cancer therapy,[7] for anti-aging,[8] as an immunostimulatory agent[9] or as a sleep regulator,[10] as well as to increase bone density in menopausal patients[11] **(Fig. 1)**. It is reported that salivary melatonin is released by the acinar cells of the major salivary glands and the gingival crevicular fluid. It follows a circadian rhythm, with the highest values at night. Moreover, in the oral cavity, melatonin can act both by receptor-mediated and by receptor-independent pathways.[12] Therefore, through complex molecular pathways that have gained special interest for the research community in periodontology, it may play a role in alveolar periodontal and periimplant bone maintenance and regeneration.

Melatonin is an amphiphilic molecule that is able to cross most biological barriers. It can exert its effect by binding to G-protein-coupled membrane receptors (MT_1 and MT_2) or by penetrating the cell through a specific family of transmembrane channels,[13] subsequently initiating a nuclear or cytoplasmic molecular cascade. When it reaches the nuclei, melatonin binds to a subfamily of nuclear receptors key in regulating bone metabolism, the RZR (retinoid Z receptor)/ROR (retinoid orphan receptor) receptor.[14] It then regulates a number of cellular events, such as promotion of mitosis, induction of DNA repair,[15] or cell differentiation and proliferation.[16]

Interestingly, it is known that melatonin can be synthesized in the bone marrow, where its concentration is around 100-fold higher than in serum.[17] Furthermore, melatonin in the bone marrow protects its cells against cytotoxic agents *in vivo*.[18] However, the specific biochemical mechanisms that regulate this modulation, specifically in alveolar bone in humans, are currently not fully understood.[11] Hence, it is the purpose of this review to describe the properties of melatonin in regulating bone homeostasis, directly and indirectly, as well as to analyze different therapeutic strategies for the use of melatonin as an agent to promote periodontal and periimplant bone maintenance and regeneration **(Fig. 2)**.

Direct effects on bone

I. Melatonin and bone formation

The major organic component of bone extracellular matrix is Type I collagen, which supports the expression of bone cell phenotypes and enhances mineralization. Melatonin has been shown to regulate the synthesis of Type I collagen as a preliminary step to the expression of other bone-related proteins, such as bone sialoprotein, alkaline phosphatase and osteocalcin, during osteoblastic maturation.[16]

Bone sialoprotein (BSP) is referred to as a marker of the late stage of osteoblastic differentiation. BSP is expressed during osteoblastic cell differentiation in the extracellular matrix, where it is essential for osteoblast attachment and bone mineralization. Within this context, it has been reported that MC3T3 pre-osteoblast cells matured in 12 days in the presence of melatonin compared with 21 days without melatonin. Gene expression of BSP and related proteins of osteoblastic differentiation (e.g., osteocalcin, alkaline phosphatase) is also accelerated and increased in melatonin-treated compared with nontreated cells.[19] Furthermore, by inhibiting the interaction of BSP with osteoblastic cell lines, the activity of alkaline phosphatase, osteocalcin synthesis and cellular response to parathyroid hormone (PTH) are also inhibited[20] and, subsequently, osteoblast differentiation is impaired.[21] Thus, these findings suggest that melatonin may have an effect in regulating osteoblast proliferation and differentiation. These effects could lead to beneficial effects in the treatment of pathological processes associated with bone resorption or destruction by mediating not only in the expression of BSP but of other bone glycoproteins as well, resulting in enhanced bone apposition.

II. Melatonin and bone resorption

Melatonin also exerts an important direct biological action on the osteoclast, another key cell in

Fig. 1

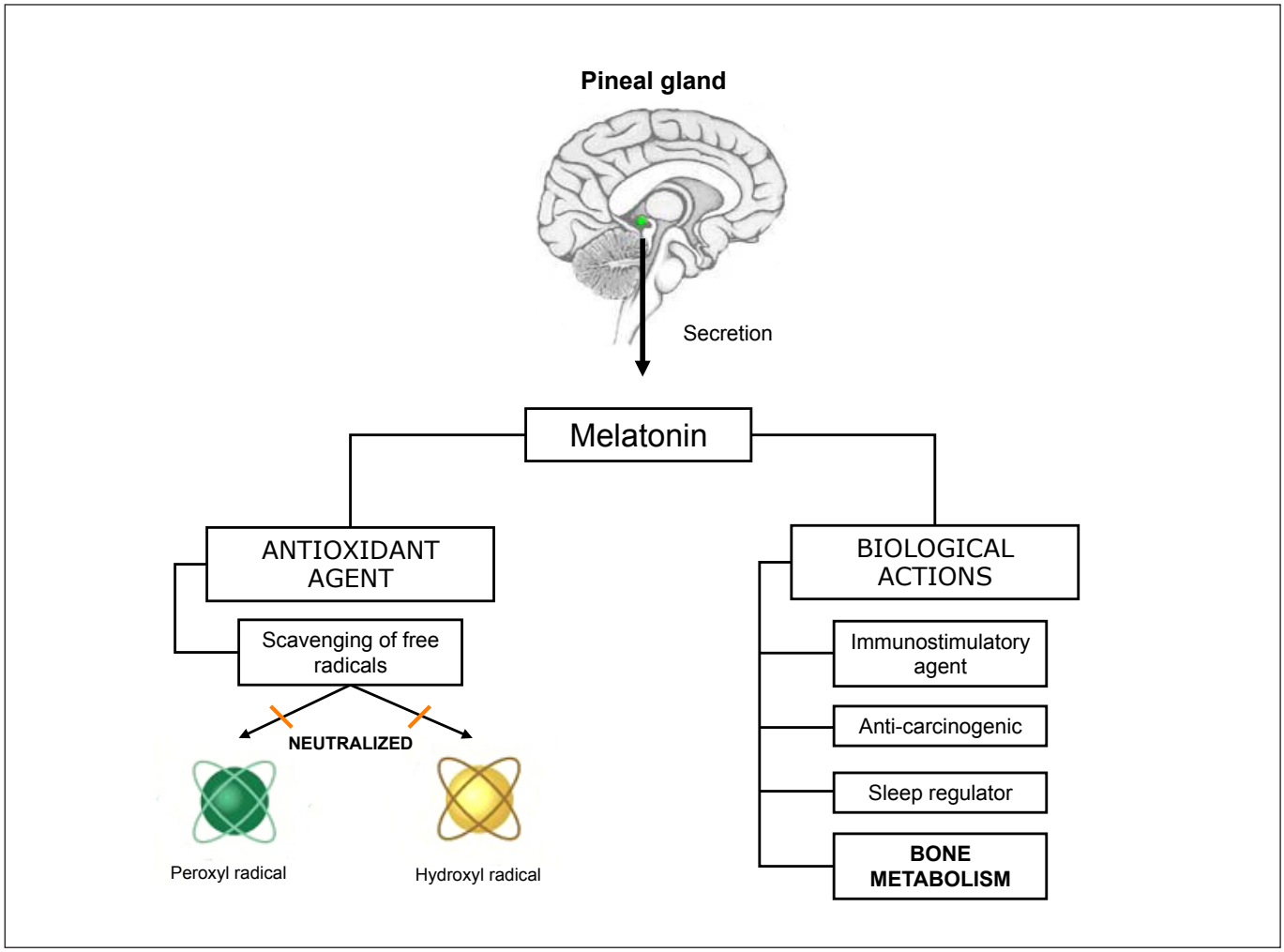


Fig. 1

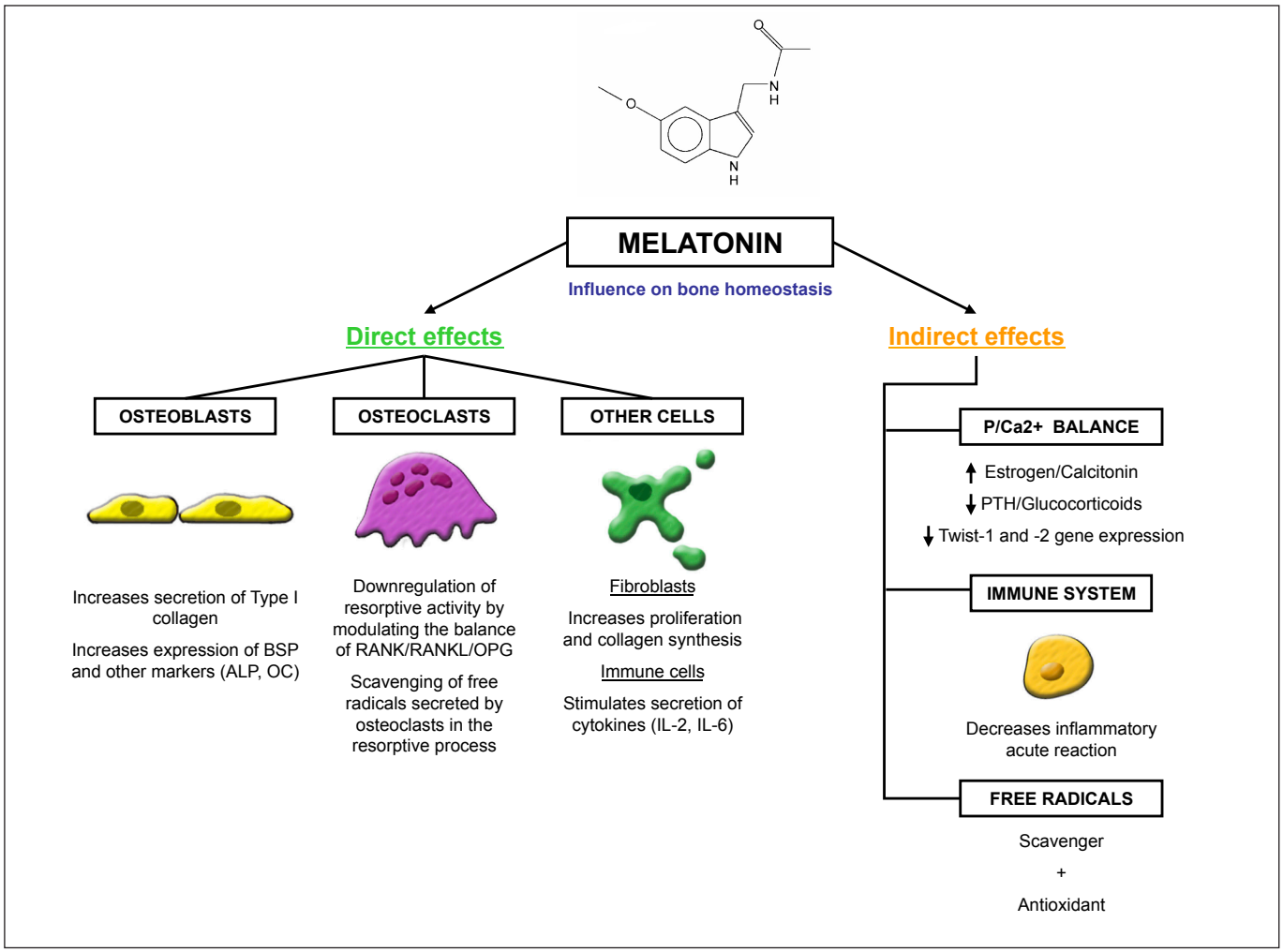
Melatonin properties.

bone turnover. The biological activity of osteoclasts is bone resorption, initiated by attachment to the surface of the bone tissue and secreting protons and free radicals into the cell compartment formed below their ruffled border. The activity of osteoclasts is essentially regulated by the molecular triad osteoprotegerin/receptor activator of nuclear factor-kappa B/receptor activator of nuclear factor-kappa-B ligand (OPG/RANK/RANKL). The balance and expression of this triad in bone tissue decisively influence differentiation and activation of osteoclasts and play an important role in coordinating osteogenesis, odontogenesis and tooth eruption. The proteins of this triad can be synthesized by a large number of cells, including bone marrow cells, dendritic cells, lymphoid cells and endothelial cells. Osteoblasts are the key cell type in the secretion of OPG and RANKL and, therefore, orchestrate the bone turnover. Changes in the expression balance of this triad can be responsible for hereditary bone disorders, such as familiar expansile osteolysis, expansile skeletal hyperphosphatasia and juvenile Paget's disease; different forms of osteoporosis; and other metabolic bone diseases.

The OPG/RANK/RANKL triad can be modulated by numerous molecules, including melatonin.[22] Melatonin suppresses osteoclastic and osteoblastic activity by interacting with this triad.[23] It reduces the expression of RANK in osteoblasts[24] and RANK receptor in osteoclasts[19] while increasing OPG,[24] eventually preventing the appearance and activation of osteoclasts.[19] This suggests that melatonin in pharmacological doses can inhibit bone resorption and increase bone mass by down-regulating RANK-mediated osteoclast proliferation and activation.[24]

Another important aspect of the relationship between osteoclasts and melatonin concerns the production of free radicals by osteoclasts during osteolysis. Osteoclasts generate high levels of superoxide anions during bone resorption that contribute to the degenerative process of the organic bone matrix. One of the most important

Fig. 2



mechanisms underlying this resorption involves the protective superoxide-scavenging enzyme superoxide dismutase. Melatonin is a significant free-radical scavenger and antioxidant at both physiological and pharmacological concentrations.[6] Beside its ability to directly neutralize a number of free radicals and reactive oxygen and nitrogen species, melatonin stimulates several antioxidative enzymes,[19,25] limiting the resorptive osteoclast activity.

Indirect effects on bone

1. Relationship with hormones and genes involved in bone turnover

Melatonin is an important modulator of calcium and phosphorus metabolism.[26] In addition to its direct actions on cells that modulate bone homeostasis, melatonin may exert its effects indirectly on the bone system by influencing the activity of important regulators of the phosphorus–calcium balance and bone metabolism. Many studies have indicated that melatonin may influence the release of several factors that affect bone, such as calcitonin,[27] corticosterone,[28] growth factors[29] and immunological factors.[30]

- Calcitonin, together with bisphosphonates and estrogens, is an important regulator of the apoptosis of osteoclasts. It is a powerful inhibitor of osteoclastic resorptive activity by promoting the reduction of contact between osteoclasts and the bone surface, altering the morphology of osteoclasts and decreasing their mobility. Melatonin increases secretion of calcitonin in rats, and this may inhibit bone resorption.[31]
- PTH increases the expression of RANKL and decreases the expression of OPG. Melatonin decreases the levels of PTH, and this may, indirectly, generate an increase in bone mineralization.[32]

Fig. 2

Effects of melatonin on bone. ALP: alkaline phosphatase; OC: osteocalcin.

– Cortisol (also known as hydrocortisone) and other glucocorticoids are increased when melatonin is reduced. They are responsible for inhibiting bone formation through direct actions on osteoblasts by blocking their recruitment and differentiation, and subsequently inhibiting the production of Type I collagen. An increase in cortisol is also responsible for an increase in bone resorption via antagonism of the 1,25-dihydroxyvitamin D. Therefore, as melatonin increases, glucocorticoids are reduced and their pro-resorptive effects are limited.
– Melatonin also stimulates estrogen secretion and, therefore, limits the associated deleterious effects of deficiency.[33]

II. Action in immune system

The role played by melatonin in the immune system is well documented.[34] The effects of melatonin have been most widely studied in the context of depressed immune systems with the aim of improving immunodeficiency situations. Melatonin regulates the apoptosis of B and T cells and has been reported to accelerate the production of leukocytes.[35]

In addition to the direct effect on cells of the immune system, melatonin reduces the synthesis of prostaglandins, especially PGE-2; prevents the translocation of nuclear factor-kappa B to the nucleus and its binding to DNA, thereby reducing the up-regulation of a variety of pro-inflammatory cytokines;[36] inhibits the production of adhesion molecules that promotes the adhesion of leukocytes to endothelial cells;[37] and attenuates transendothelial cell migration and edema, which contribute to tissue damage.[30] It also stimulates the release of interleukin-2 in Jurkat cells[38] and interleukin-6 in peripheral blood mononuclear cells,[39] while it inhibits the inflammatory enzyme cyclooxygenase-2 (COX-2) and binds to the active sites of COX-1 and COX-2.[40] Therefore, melatonin can inhibit acute inflammatory reaction and contribute to generating an immune reaction, minimizing the associated bone loss.[30]

III. Action on free radicals

One of the principal biological actions of melatonin is its wide antioxidant spectrum and powerful endogenous effect as a free-radical scavenger.[41] Thus, it has an indirect reparative effect and prevents intracellular damage, protecting cells from free radicals and chemical substances. Melatonin acts on oxygen- and nitrogen-derived free radicals, including the highly toxic hydroxyl radical,[42] peroxynitrite anion[43] and hypochlorous acid.[44] In addition to directly neutralizing free radicals and reactive species of nitrogen and oxygen, melatonin stimulates other antioxidant enzymes, such as glutathione.[45]

At the bone level, these effects are of vital importance because osteoclasts secrete a wide variety of molecular agents for bone degradation. Free radicals are the highly secreted ones. Osteoclasts generate superoxide anions during resorption that contribute to the degradative processes of the organic bone matrix. Other cell types, such as monocytes, macrophages and neutrophils, accumulate on the adjacent surfaces of the bone in chronic inflammatory processes. These cells have the capacity to produce free radicals and, as previously mentioned, are able to stimulate osteoclastic response by liberating mediators (cytokines, tumor necrosis factors, etc.). Therefore, the use of anti-free-radical agents might be an adequate alternative therapy for these types of pathologies, by limiting osteoclastic activation or free-radical production.

Melatonin and periodontal disease

Periodontal disease is caused by a bacterial challenge that triggers an inflammatory reaction in a susceptible host. Alterations in the OPG/RANK/RANKL complex, among other cytokines and local factors, have been linked to an increase in the periodontal destruction, mediated by the increase in RANKL production by inflammatory cells, mainly macrophages, and the decrease of OPG. Additionally, the periodontal tissue is affected by the free radicals that burst from phagocytic cells, such as neutrophils and macrophages, which significantly damage the gingival tissue.

In view of these common factors and targets, it is reasonable to expect an association between periodontal disease and levels of melatonin.[46] Several clinical studies have demonstrated it.[3,4,47] These studies showed that levels of melatonin in serum, saliva, gingival crevicular fluid or all three are inversely associated with the severity of the disease, which indicates that melatonin may have a protective role against periodontal disease.

Moreover, the effects of melatonin on the reduction of osteoclastogenesis, the capture of reactive oxygen species and their metabolites in the inflamed area, the increase in bone minera-

lization through the increase in proliferation, differentiation and activity of osteoblasts, and collagen and BSP regulation, as already explained, together with its anti-fibrotic and anti-inflammatory effects on gingival tissue by a reduction of the matrix metalloproteinase-1/tissue inhibitor of metalloproteinases-1 ratio, suggest the possibility of using melatonin as a host-modulating agent in the treatment and control of periodontal disease, improving the bone tissue conditions and the soft-tissue stability.[48] The *in vivo* administration of local or systemic melatonin could, therefore, be indicated in these patients, although no studies have yet been performed in this sense with validated methods.

Melatonin and dental implants

Dental implants are commonly used in current treatment of tooth loss. However, to avoid potential early complications and implant failures,[49, 50] bone healing must occur in the proper way. Bone remodeling around dental implants is highly influenced by the implant surface characteristics and evolves as a balance between the activity of osteoblasts and osteoclasts.[51, 52] Therefore, the use of melatonin as a topical agent to induce biomimetic properties of the implant surface has emerged as a promising technique.[53] Melatonin directly influences the osteoblast's response to the implant surface and osseointegration. The addition of melatonin improves results for cell adhesion, proliferation and differentiation on different titanium surface modifications at early time points, although longer culturing times seem to reduce those differences.[54]

These effects have been confirmed *in vivo* in several studies.[55, 56] The effects around dental implants are similar to those that take place in bone repair. Bone repair consists, biologically, of three different stages: inflammatory, proliferative and remodeling. Melatonin may play a role in these phases owing to its regulatory effects on inflammation, antioxidant properties, regulation of bone cells, and stimulation of collagen synthesis and deposition. Moreover, melatonin has been shown to increase the number of blood vessels, which is a prerequisite for the supply of mineral elements and the migration of angiogenic and osteogenic cells. As a consequence, histological evaluation of the periimplant bone shows more trabecular bone, but less cortical bone and higher bone-to-implant contact in melatonin-treated sockets compared with controls.[55] Therefore, the use of melatonin for osseointegration might be of interest as a biomimetic agent. Moreover, it has been suggested to induce bone growth when applied in combination with bone grafts.[56] However, its potential use in regenerating post-periimplantitis defects has not been studied yet.

Conclusion

Melatonin positive regulation of bone formation and homeostasis, in combination with the inhibitory effects on bone resorption, highlights the potential use of melatonin as a marker of periodontal and periimplant bone-related diseases. Moreover, *in vitro* and animal studies are starting to show promising results on its use as a regenerative agent, although no clinical studies have yet been performed. This new strategy may create possibilities for novel therapies in the treatment of periodontal disease or enhancing the outcomes of implant dentistry.

Competing interests

The authors declare that they have no conflict of interests related to this study.

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