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# 13 Melatonin in Dentistry

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## 13.1 MELATONIN

Melatonin is an indolamine hormone (*N*-acetyl-5-methoxytryptamine) synthesized from the essential amino acid tryptophan in a series of four enzymatic steps.<sup>1,2</sup> It was originally discovered in 1958 by the American dermatologist Aaron Lerner and his coworkers at the Yale University School of Medicine as an amphibian skin-lighting factor present in extracts of bovine pineal glands. Lerner named the molecule “melatonin” because it induces contraction of stellate amphibian melanophores.<sup>3</sup> The fact that melatonin is an evolutionarily highly conserved molecule, ubiquitously distributed in living systems, seems to demonstrate that it has important physiological roles.<sup>4</sup>

### 13.1.1 BIOSYNTHESIS, SECRETION, AND METABOLISM

In vertebrates, melatonin is primarily secreted by the pineal gland, a neuroendocrine transducer considered until the first half of 20th century as an epithalamic appendage of brain with enigmatic functions;<sup>5,6</sup> therefore, there are also extrapineal sites of melatonin production, on which it exerts a local action. However, with the exception of the retina and the GI tract (in which melatonin is stored)<sup>7</sup>, the physiological significance of these extrapineal sites is still a matter of debate. In the pineal gland, melatonin is synthesized by pinealocytes, whereas in the retina, it is produced by

photoreceptor cells.<sup>8–10</sup> Melatonin produced by the pineal gland is immediately released into the blood vascular system and cerebrospinal fluid of the third ventricle where exerts various biological actions upon reaching melatonin receptor-rich target tissues; melatonin secreted by the retina instead is not released into the circulation and so exerts a paracrine function.<sup>11</sup> Once melatonin is released into the circulation (where 50%–75% of melatonin is bound reversibly to albumin and glycoproteins), it has a short half-life:<sup>4</sup> most of the circulating melatonin (95%) is primarily metabolized in the liver by cytochrome P450 enzymes; alternatively, it is metabolized by oxidative pyrrole-ring cleavage<sup>12</sup> in a number of extrahepatic tissues by both enzymatic and nonenzymatic mechanisms.<sup>13</sup>

### 13.1.2 MECHANISM OF ACTION

Although the mechanisms by which melatonin modulates the pathways in which it is involved are currently not completely understood, it has been demonstrated that it acts on its target cells/tissue through transmembrane G-protein-coupled receptors MT1, MT2, and MT3 or through orphan nuclear receptors of the retinoic acid receptor family.<sup>14</sup> However, in other particular biological contexts, no specific receptors appear to be required for melatonin's actions;<sup>15,16</sup> being a lipophilic molecule, it has the capacity to pass freely in and out of all cellular and fluid compartments of the body having free access to all cells of the body and thereby offers an additional receptor-independent nonhormonal role of free radical scavenger in reducing the oxidative stress.<sup>13</sup>

### 13.1.3 MAIN BIOLOGICAL FUNCTIONS

Studies in various animals, especially in mammals, have shown that, at either physiological or pharmacological concentrations, melatonin appears to be involved in numerous physiological and pathophysiological processes including the control of sleep, circadian rhythms, retinal physiology, seasonal reproductive cycles, cancer development and growth (melatonin has oncostatic potentials), regulation of the immune response,<sup>17,18</sup> antioxidation, and free radical scavenging (up-regulating several antioxidative enzymes and down-regulating pro-oxidant enzymes),<sup>19</sup> mitochondrial respiration, cardiovascular function and blood pressure control,<sup>20,21</sup> bone metabolism, and gastrointestinal physiology.<sup>13,22</sup> Other actions of this hormone include the inhibition of dopamine (DA) release in the hypothalamus and in the retina,<sup>23</sup> and it is also involved in pubertal development<sup>24,25</sup> and the aging process.<sup>26,27</sup> On this purpose, it has been remembered that the amount of melatonin produced during the night appears to be greatest around the time just before puberty, with a steady decrease thereafter through middle and old age. For this reason, the decrease in melatonin production has implications for various diseases, including age-associated neurodegenerative diseases and cancer initiation.<sup>28</sup>

## 13.2 MELATONIN IN THE ORAL CAVITY

In the following pages, we will report the specific roles that melatonin exerts at the oral cavity level, both in physiological and in pathological conditions. In particular, the purpose of this review is to explain the potential utility of melatonin in the treatment of the most relevant pathologies affecting the oral cavity: tooth decay, periodontal diseases (PDs), and oral cavity cancer-associated inflammation.

### 13.2.1 MELATONIN IN THE ORAL CAVITY OF HEALTHY SUBJECTS

Saliva may be described as a heterogeneous fluid composed of proteins, glycoproteins, electrolytes, and small organic molecules, as well as compounds transported from the blood.<sup>29</sup> It represents a combination of gingival crevicular fluid (similar to serum for its composition) and other fluids released from salivary glands, of which the parotid, submandibular, and sublingual are the three

major sources.<sup>30</sup> The secretion of saliva has been shown to be affected by different forms of stimulation: time of day, diet, age, sex, a variety of disease states, and several pharmacological agents.<sup>31</sup>

Once in the blood, melatonin gets to the saliva by passive diffusion. The ratio between salivary and plasmatic melatonin in a (24-hour) cycle is relatively stable and varies from 0.24 to 0.33: it means that the salivary melatonin concentration is equivalent to 24%–33% of the plasma levels.<sup>32,33</sup> As reported above, the amount of melatonin in saliva is lower when compared with that of blood probably due to the fact that the major part of the plasma melatonin is bound to albumin (nearly 70%) and so it is not free to diffuse into saliva. Thus, salivary melatonin reflects in appreciable extent the portion of circulating melatonin not bound to proteins (the free-circulating melatonin).<sup>34</sup> It is possible that melatonin is also produced and released locally by the mucosal lining of the oral cavity, but this still requires direct experimental evidence. Salivary melatonin can be reliably assayed. By measuring salivary melatonin, oral pathologies can be studied in relation to plasma and salivary melatonin behavior. A significant positive correlation between salivary and plasma melatonin exists: according to Konturek et al.,<sup>35</sup> the concentration of salivary melatonin under basal conditions is negligible, but following local oral application of indoleamine, its plasma level dose-dependently increases, and this is consequently accompanied by an increase of salivary melatonin.

Melatonin has several specific functions in the oral cavity, so its effects on oral health warrant further investigations. It acts as a potent antioxidant and free radical scavenger, immunomodulatory agent, strong promoter of bone formation, and anti-inflammatory factor in PDs.<sup>36</sup> Recently, it has been claimed that the imbalances in levels of free radicals and reactive oxygen species (ROS) with antioxidants may play an important role in the onset and development of several inflammatory oral pathologies.<sup>37</sup> On this purpose, current evidences for oxidative damage in the most prevalent oral cavity diseases and the possible therapeutic effects of antioxidants like melatonin<sup>38</sup> have been extensively reviewed in the last years.

### 13.3 MELATONIN AND ORAL CAVITY DISEASES

#### 13.3.1 TOOTH DECAY

Dental caries, also known as tooth decay, is the localised destruction of susceptible dental hard tissues by acidic by-products from bacterial fermentation of dietary carbohydrates.<sup>39,40</sup> Tooth decay can affect the enamel (the outer covering of the crown), cementum (the outermost layer of the root), and dentine (the tissue beneath both the enamel and the cementum). As partially reported above, dental caries results from interactions over time between the products of bacteria metabolism and many host factors that include teeth and saliva. So, even if dental caries is a multifactorial disease (related, for instance, to high numbers of cariogenic bacteria, insufficient fluoride exposure, gingival recession, immunological components, need for special health care, and genetic factors)<sup>39,41</sup> resulting from an ecological imbalance in the physiological equilibrium between tooth minerals and oral microbial biofilms,<sup>42</sup> it is greatly affected by salivary flow and composition and also by dietary habits.<sup>43</sup>

Given the presence of melatonin in the oral cavity because of its release in saliva<sup>11</sup> and given its beneficial properties, it has been hypothesized that this hormone could have a cariostatic potential. On this purpose, with the strong influence of diet on cariogenesis, Mechin and Toury<sup>44</sup> suggested that, melatonin, being abundantly available in the foodstuffs, may diminish the caries development with its antioxidant activity. In their study, Mechin and Toury<sup>44</sup> tested the effects of melatonin administration on caries development in rats receiving a standard or a modified cariogenic diet 2000M: a large increase in the caries score was induced by the cariogenic diet as compared with the control group. Moreover, in groups receiving melatonin, a highly significant decrease in the caries score was obtained. However, the explanation of these results can only be speculative. In fact, no antibacterial action was attributed to melatonin, and there are no evidences about the possible action of melatonin on calcium metabolism and on the mineralization/demineralization

equilibrium. Nevertheless, the hypothesis of the possible action of melatonin on various salivary components that can modify the cariostatic potential of saliva is more intriguing and needs further investigation.

### 13.3.2 PERIODONTAL DISEASE

PD is an oral inflammatory disorder of the periodontium that affects the supporting tissues of the teeth (alveolar bone, gums, and periodontal ligament), leading to progressive destruction of connective tissue attachment and alveolar bone. A consequence is the severe loss of supporting periodontal tissues and teeth, seen prevalently among adults and older people. Current information indicates that bacterial infection and accumulations on the teeth may be the primary causative agent of PD.<sup>45,46</sup> Nowadays, PD represents one of the most commonly reported chronic inflammatory adult conditions. Approximately 48% of U.S. adults have chronic PD, and similar or higher rates (up to 70%) have been reported in other populations.<sup>47</sup> PD incidence is increased by several risk factors; in general, all those conditions that provide the anaerobes ample time to survive in periodontal tissue or any medical conditions (e.g., HIV infections) that trigger host antibacterial defense mechanisms will likely promote PDs.<sup>48</sup>

The severity of periodontitis is characterized by the degree of marginal bone loss, depth of periodontal pockets, degree of attachment loss, and number of teeth with furcation development.<sup>49</sup> In diagnosing PD, the probing depth is a good indicator of the advance of the disease. In a healthy periodontium, there is no loss of epithelial attachment or pocket formation and the periodontal pocket is less than 2 mm deep.<sup>50</sup> The disease state ranges from gingivitis to periodontitis and advanced periodontitis.

Gingivitis, the most prevalent and mild form of PD, is characterized by the inflammation of the gums caused by plaque deposits, with possible bleeding when brushed or probed.<sup>49</sup>

Periodontitis can be identified by the hardening of plaque to form calculus, causing gum recession. This results in the formation of pockets between 3.5 and 5.5 mm between the tooth surface and the gum.<sup>51</sup> The symptoms are similar to those of gingivitis but are more severe due to higher accumulation of bacteria and stronger inflammatory responses.

Advanced forms of periodontitis are also prevalent, affecting approximately 10%–30% of the adult population in the United States.<sup>52</sup> Advanced periodontitis is distinguished by excessive tissue loss of gingiva and alveolar bone and pockets greater than 5.5 mm in depth. This condition often leads to tooth exfoliation due to the destruction of the tooth connective ligaments.<sup>53</sup>

The etiology and pathogenesis of PD are not completely clear. Human gingivitis and periodontitis are the results of an imbalance in the bacterial species that colonize the oral cavity and are characterized by complex interactions between pathogenic bacteria and the host's immunoinflammatory responses.<sup>55,56</sup> In the past three decades, marked advances have occurred in our understanding of the infectious agents of PD. There are more than 300 distinct species of bacteria present in the gingival area of the mouth, most of which exist in a commensal relationship with the host. However, three Gram-negative, anaerobic, or microaerophilic bacteria species, known as periodontal pathogens (*Actinobacillus actinomycetemcomitans*, *Bacteroides forsythus*, and *Porphyromonas gingivalis*), have been identified as being ubiquitous in periodontal plaque formations.<sup>53,54,56</sup> Moreover, within the past years, various herpes viruses, such as human cytomegalovirus and Epstein–Barr virus, have also emerged as pathogens in the destructive PD.<sup>57</sup>

As reported above, the damage of periodontal tissues results both from a direct effect of the toxic products released by the bacteria and from the action of the immune system that, if stimulated by bacterial infection, produces and releases mediators that induce the effectors of connective tissue breakdown.<sup>58,59</sup> Numerous studies have showed that the destruction of periodontal tissue in PD is mainly due to host-derived mediators and free radicals.<sup>60,61</sup> Different mechanisms, including DNA damage, lipid peroxidation, protein damage, oxidation of important enzymes, and stimulation of proinflammatory cytokine release, have been implicated as causes of tissue damage by an increase

in both ROS and reactive nitrogen species (RNS).<sup>62,63</sup> An inverse relationship between peroxidation products and antioxidant molecules or enzymes in spontaneous or in experimental PD has been stressed.<sup>64–66</sup> Chapple et al.<sup>53</sup> reported that total antioxidant activity is reduced in saliva of patients with periodontitis relative to that in nonperiodontitis subjects. The imbalance between oxidative stress induced by ROS and the concentrations (or activity) of the antioxidants may lead to a further oxidative attack and substantial deterioration of the periodontal tissues,<sup>67,68</sup> resulting in tissue damage.<sup>69,70</sup>

Microbial components, especially lipopolysaccharide (LPS), have the capacity to induce an initial infiltrate of inflammatory cells. Activated macrophages synthesize and secrete a variety of proinflammatory molecules, including some interleukins (IL-1 $\alpha$ , IL- $\beta$ , IL-6, and IL-8), tumor necrosis factor  $\alpha$  (TNF- $\alpha$ ), prostaglandins (PGE2), and hydrolytic enzymes.<sup>71</sup> These cytokines recruit polymorphonuclear leukocytes (PMN) to the site of infection.<sup>54</sup> PMN play a relevant role in the etiology of PD, as they are the predominant host immune response to oral bacterial infection. Upon stimulation by bacterial antigens, cytokines promote the PMN to express adhesion molecules and move out of the circulation to the site of infection.<sup>72</sup> When PMN arrive here, they can induce an autoamplification effect producing IL-8 to attract more PMN into the infection site. This is exacerbated by the ability of *P. gingivalis* to modulate the mobility and function of PMN within the site of infection:<sup>54</sup> a reduction of IL-8 secretion in epithelial cells, mediated by the bacterium, inhibits the recruitment of PMN to the infected area. At the site of infection, PMN produce proteolytic enzymes (e.g., elastase), but also ROS. Indeed, PMN in periodontal patients display an increased number, adhesion, and oxidative activity.<sup>73</sup> As the release of ROS is not target-specific, damage to host tissue also occurs. Gingival epithelial cells are highly susceptible to attack by PMN-derived oxidants;<sup>74</sup> human PMN produce *in vitro* desquamation (as a consequence of the digestion of extracellular matrix constituents by PMN neutral proteases) and lysis of gingival epithelial cells (caused by PMN oxidants generated by myeloperoxidase).<sup>38</sup>

In PD, a number of proteases that degrade collagen and extracellular matrix (ECM) play key roles in periodontal tissue breakdown.<sup>75</sup> A particular subgroup of matrix metalloproteinases (MMPs), called collagenases, is the major group of enzymes responsible for degradation of ECM and for collagen destruction in periodontitis. These latent collagenolytic enzymes are activated by ROS in the inflammatory environment, giving rise to elevated levels of interstitial collagenase in inflamed gingival tissue.<sup>76</sup> The attachment loss deepens the sulcus, creating a periodontal pocket. This pocket provides a microbial niche that can harbor on the order of 100 bacterial cells.<sup>77</sup> This event marks the transition from gingivitis to periodontitis.

PD is clearly an important and potentially life-threatening condition, often underestimated by health professionals and the general population. The available evidence implicating inflammatory mediators and cells in the disease process suggests that local antioxidant status may be of importance in determining susceptibility to the disease and its progression following initial bacterial colonization.

### 13.3.2.1 Gold Standard Therapies in PD

Due to the minimal symptoms of gingival bleeding and attachment loss, many individuals neglect to treat their disease. Left untreated, gingivitis may progress to irreversible periodontitis, resulting in tooth loss.

Periodontal research has provided sufficient evidence indicating that, once diagnosed, chronic PD is successfully treatable.<sup>78</sup> The first therapeutic goal in treatment of PD is to alter or eliminate the origin of the microbes as well as the contributing risk factors. The majority of periodontal treatment modalities, however, attempt to arrest the progression of periodontal destruction in order to avoid tooth loss and preserve the healthy state of the periodontium.<sup>79</sup> Furthermore, in severe cases, regeneration of the periodontal attachments must be attempted.<sup>80</sup> The first nonsurgical step of PD treatment involves special cleaning called scaling and root planing. Supplemental treatment may include antiseptic mouth medications, either to aid the healing process or to further control the



bacterial infection. Often, antibiotics may be administered, which may offer an effective alternative. Doxycycline, a wide-spectrum antibiotic, and other tetracyclines are frequently used in dental treatments for soft tissue and bone regeneration after PD because of their strong activity against periodontal pathogens; they are able to inhibit the activity of human MMPs and reduce the severity and progression of PDs in animal models and humans.<sup>81</sup> Along with antibiotic therapy, if the periodontal pockets are not reduced or further loss of alveolar bone is observed, surgical treatment may therefore be beneficial to PD patients to prevent bone loss. If the PD has caused excessive loss of gum tissue or bone, then soft-tissue grafts or bone grafts may be performed to reduce further gum recession and bone loss.

### 13.3.2.2 New Perspectives in PD Treatment: Melatonin Supplementation

In recent years, the role of ROS, lipid peroxidation products, and antioxidant systems in the pathology of PD have been well clarified. It is now of importance to determine the possible contribution of diet to salivary antioxidant status because the use of antioxidant supplementation in the treatment or prevention of these chronic diseases of the oral cavity can be an excellent chance. Recent medical and dental research in this area is geared toward the prevention of free radical-mediated diseases by using specific nutrient antioxidants supplementation.<sup>38</sup> Melatonin was found to be released with saliva into the oral cavity and to be implicated in various dental and PDs: for this reason, it is one of the more prominent antioxidant used on this purpose. In particular, melatonin possesses two functions of great interest to dental professionals: first of all, its capacity to scavenge free radicals, thereby exerting antioxidative action,<sup>82,83</sup> and second, the cell protective effect exerted by melatonin in situations of inflammation.<sup>37,84</sup>

Nowadays, it is well known that melatonin not only would stimulate the immune system through the plasma fraction of the hormone but would also afford local protection through the salivary melatonin fraction<sup>85</sup> to better protect the cell populations affected by the periodontal process from the ROS generated by the inflammatory process. Saliva antioxidant capacity was significantly lower in diseased patients compared with controls. In addition, the ratio between saliva and serum antioxidants was also significantly lower in the diseased patients. It was proposed that the reduction in antioxidant capacity was either a direct causal factor in the PD patients or that the reduction was due to a reduction in scavenging antioxidants mediated through an increase in oxidative stress due to the pathogenesis of the disease. Cutando et al.<sup>85</sup> emphasized the physiological impact of melatonin in saliva: this little amine displays noticeable antioxidant activity in saliva<sup>86</sup> and helps protect the oral cavity from tissue damage due to oxidative stress. In a recent study, it was indirectly shown that nitric oxide (NO) production was elevated in the diseased periodontium. In ligature-induced periodontitis in rats, inducible nitric oxide synthase (iNOS) was expressed at higher concentrations at the ligated sites than at the nonligated sites.<sup>87</sup> The diseased tissue biopsies from periodontitis patients demonstrated a greater level of iNOS expression than the healthy tissue biopsies from the clinically nonperiodontitis patients. In particular, the basal layers of epithelium and macrophages, lymphocytes, and neutrophils in the connective tissue were found to stain positively for iNOS, only in diseased patients.<sup>88</sup> Recent evidence suggests that the pineal hormone melatonin, acting as a potent free radical scavenger, plays an important acute and chronic role in reducing or eliminating the oxidant damage produced by NO.<sup>89,13</sup> Based on a number of studies, it is estimated that melatonin inhibits the activity of NOS,<sup>90,91</sup> in particular iNOS, which produces excessive amounts of NO, thus contributing to the pathophysiology of inflammation and increasing the oxidative stress.<sup>92</sup>

Furthermore, in PDs, the increase in free radical production coexists with a decrease in antioxidant defense. Besides its direct action as a free radical scavenger, melatonin influences the oxidative stress status indirectly by stabilizing the inner mitochondrial membrane and improving the electron transport chain located there.<sup>93</sup> It has been demonstrated that melatonin is a broad-spectrum antioxidant:<sup>94,95</sup> in pharmacological and physiological doses, it increases gene expression and activity of endogenous antioxidant, which are important in maintaining the integrity of vasculature and other tissues.<sup>96,97</sup> These antioxidant properties of melatonin could turn out very beneficial for

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treatment of the local inflammatory lesions and for accelerating the healing process (e.g., after tooth extraction and other surgical procedures in the oral cavity). Recently, Cutando et al.,<sup>98</sup> have shown the favorable effects of the local melatonin administration to the alveolar sockets after molar or premolar extraction in dogs. The animals without melatonin regimen showed an increase in lipid peroxidation, nitrite plus nitrate levels in plasma, and glutathione disulfide/glutathione ratio. Dogs who were administered 2 mg melatonin to the extraction socket just after extraction did not show this increase.<sup>98</sup> This suggests that locally applied melatonin to the oral cavity lining may be useful in the treatment of oral lesions.<sup>99</sup> Czesnikiewicz-Guzik et al.<sup>99</sup> attempted to measure the concentrations of melatonin in the saliva and plasma of patients after application of melatonin to restricted areas of oral mucosa, such as the palate. It was found that melatonin is quickly and in a time-dependent fashion absorbed into the circulation from the mucosa of the oral cavity as documented by the increment of plasma immunoreactive melatonin levels that was paralleled by the increase in salivary concentrations of this indole. These results may have important clinical implications because melatonin could be applied directly on oral mucosa in a variety of infectious and noninfectious oxidative stress diseases of the oral cavity including denture-induced stomatitis, gingivitis, healing of lesions, and ulcerations caused by tooth extraction (alveolitis). Moreover, these studies indicate that topically applied melatonin to the oral mucosa in the area of damage or inflammation is effective in combating the inflammatory processes and acceleration of the healing of erosions and ulcerations in the oral cavity.

Furthermore, melatonin seems to have also a direct effect above the cell populations of the immune system. It is known, for instance, that the metabolic products of periodontopathic bacteria decrease cytokine production including IL-2.<sup>100,101</sup> IL-2 regulates a series of processes in different cells of the immune system. A relationship between IL-2 and melatonin was described when it was found that melatonin stimulates the production of IL-2 by T lymphocytes.<sup>102</sup> On the other end, IL-2 can modulate the synthesis of melatonin at the level of the pineal gland.<sup>103</sup> Without doubt, this reciprocal modulation have important consequences at the time of treatment of periodontal patients who have, in one way or another, an altered immunological system. Thus, it was of interest to study the changes in the relationship between melatonin and IL-2 during periodontal pathologies. Moreover, earlier studies<sup>104,105</sup> showed that an increase in salivary and plasma melatonin resulted in stimulation of the CD4+ T cells, which possess membrane and nuclear receptors for the hormone.<sup>102</sup> This would stimulate the other immune cell populations via cytokine secretion (e.g., CD3+, CD19+, CD8+ cells), thereby facilitating the host reaction to an existing oral infection.<sup>106</sup>

Such beneficial effects of melatonin could open new perspectives for the treatment of oral inflammatory processes,<sup>85,86</sup> suggesting that this indole hormone could have a protective function in fighting periodontal infection. However, the relationship between PDs and melatonin level remains to be better understood.

### 13.3.3 ORAL CAVITY CANCER

Oral cavity cancer, predominantly oral squamous cell carcinoma (OSCC), is an important cancer, globally affecting 270,000 people worldwide each year.<sup>107</sup> Despite of the recent progress in the diagnosis and therapy of OSCC, the 5-year survival rate has not improved in more than two decades.<sup>108</sup> Therefore, a more comprehensive understanding of the molecular pathogenesis of OSCC is urgently needed to identify new targets for the effective therapy and to recognize the early state of OSCC or, when it is possible, premalignant lesions. The development of OSCC has been reported as one of the most important complication of a chronic inflammatory disease of the oral mucosa,<sup>109</sup> called oral lichen planus (OLP),<sup>110,111</sup> even if in OLP patients the underlying mechanisms of malignant transformation have not been clearly established.

The association of chronic inflammation with a variety of cancers, including OSCC, has been amply addressed.<sup>112–114</sup> ROS and RNS are considered to play a key role in inflammation-mediated carcinogenesis. ROS can generate DNA base alterations, strand breaks, damage to tumor suppressor

genes, and enhanced expression of proto-oncogenes.<sup>115</sup> ROS-induced mutation could also arise from protein damage and attack on lipids, which then initiate lipid peroxidation,<sup>116</sup> resulting in the transformation of normal cells into malignant cells.<sup>117</sup> Any changes in enzymatic and nonenzymatic antioxidant defense systems may break cellular equilibrium and cause damages and ultimately malignant transformation. In addition, overproduction of NO leads to generation of various RNS.<sup>118</sup> Excess of NO is produced from inflammatory cells via the expression of iNOS.<sup>119,120</sup> Chronic inflammation induced iNOS-dependent DNA damage in not only inflammatory cells but also epithelial cells, which could potentially develop into cancer.<sup>121,122</sup> Therefore, this DNA damage could possibly imply an association between the existence of OLP and development into oral cancer. Moreover, the magnitude of the inflammation damage associated to carcinogenesis depends not only on ROS/RNS levels but also on the body's defense mechanisms, mediated by various cellular antioxidants. Disruption of this delicate oxidant/antioxidant balance in the body seems to play a causative role in carcinogenesis.<sup>123</sup> Therefore, melatonin, which is a potent scavenger of the hydroxyl radical and peroxynitrate, may be useful in treating oxygen radical pathophysiology.<sup>124</sup> Parallel to that, melatonin stimulates the activity of antioxidative enzymes and inhibits pro-oxidative enzymes, thus physiologically synergizing with its direct free radical scavenging properties. For all these reasons, an inverse interaction between melatonin and carcinogenic processes is of focal importance.<sup>125</sup>

Generally, OSCC is considered to arise through the progressive accumulation of multiple genetic abnormalities that impair the functions of oncogenes or tumor-suppressor genes.<sup>126</sup> Besides genetic alterations, evidence has emerged that the DNA methylation of 5'-CpG islands has been shown to be a major cause of inactivation of tumor-suppressor genes in human OSCC.<sup>127</sup> Melatonin receptor 1A (*MTNR1A*), which encodes for one of two high affinity forms of a melatonin receptor, seems to be a candidate target involved in the etiopathogenesis of OSCC. Interestingly, expression of this gene was frequently silenced in OSCC cell lines without its homozygous loss, although it was present in normal oral mucosa, suggesting that *MTNR1A* might be inactivated epigenetically in OSCC and contribute to oral carcinogenesis. In several cancers, indeed, it has been reported that melatonin treatment or ectopic expression of *MTNR1A* has a growth suppressive effect on cancer cells *in vitro* and *in vivo*,<sup>128,129</sup> even though the intracellular mechanisms behind the antiproliferative actions of melatonin remain unclear.

### 13.3.3.1 Melatonin in Treatment of Oral Mucositis, A Complication of Chemotherapy

Oral mucositis is a consequence of the toxic effects of chemotherapeutic agents and irradiation on oral mucosa cells.<sup>130–132</sup> It is estimated that oral mucositis is a complication in 40% of patients receiving chemotherapy, more than 90% of those irradiated for head and neck cancer. This condition is frequently associated with severe pain and inflammation and can cause malnutrition, systemic infections, and low quality of life, as well as limiting chemotherapy doses. The clinical appearance of oral mucositis may range from mild discomfort and erythema to painful erythema and edema and/or ulcerations.<sup>133</sup>

The pathophysiology of mucositis is not known in detail. A complex hypothesis has been proposed to elucidate the mechanism by which mucositis develops and resolves. According to this, mucositis is a complex process, divided into four phases: an initial inflammatory/vascular phase, an epithelial phase, an ulcerative/bacteriological phase, and a healing phase.<sup>130</sup> The hypothesis speculates on the importance of the inflammatory response induced in the involved tissues by chemotherapy and ionizing radiation that occurs through the activation of intracellular and intercellular signaling pathways, regulating gene expression of specific proteins involved in immune and inflammatory processes (e.g., cytokines, adhesion molecules).<sup>134,135</sup> Of the many drugs and methods used to treat mucositis, none has been shown to be uniformly effective. Trials investigating locally and systemically applied treatments of mucositis include immunomodulatory drugs, anticholinergic drugs, cytokines, antiviral drugs, glutamine, and antioxidants.

Among the antioxidants currently under investigation, the pineal hormone melatonin could be added, as it has been claimed to have activity in the prevention of mucositis.<sup>136,137</sup> Locally and



systemically applied melatonin has been shown to prevent and treat mucositis in patients with cancer.<sup>138</sup> The pineal hormone melatonin inhibits the production of free radicals that mediate the toxicity of chemotherapy. Nevertheless, experimental data are still controversial: chemotherapy-induced stomatitis was not reduced in a study with the use of melatonin, although other toxic effects were decreased.<sup>139–141</sup> Additional basic and clinical researches are needed to determine whether melatonin can be used to treat chemotherapy-induced mucositis.

### 13.4 ROLE OF MELATONIN IN BONE METABOLISM

It is known that melatonin is involved in skeletal development: in particular, increasing evidences from *in vitro* and *in vivo* experiments using rodent and chicken have suggested the possible role of melatonin on bone metabolism.<sup>142,143</sup> The structural integrity of mammalian bone is dependent upon a balance between the activity of osteoclasts (the bone-resorptive cells) and osteoblasts (the bone-formative cells).<sup>144,145</sup> The aim of this remodeling process is the renewing of the skeleton while maintaining its anatomical and structural integrity.<sup>146</sup> Under normal conditions, bone is constantly degraded and replaced with new bone in cycles in which osteoclasts adhere to bone and subsequently remove it by acidification and proteolytic digestion. After the osteoclasts have left the resorption site, osteoblasts invade the area, and begin the process of forming new bone by secreting osteoid (a matrix of collagen and other proteins), which is eventually mineralized. After bone formation has ceased, the surface of the bone is covered by lining cells, a distinct type of terminally differentiated osteoblasts.<sup>146</sup>

Several reports indicate that melatonin is involved in the regulation of calcium homeostasis. The effects of melatonin on calcium metabolism were first studied by Csaba et al.,<sup>147,148</sup> who proposed that this hormone could influence the secretion of calcitonin<sup>149</sup> and parathyroid hormone.<sup>147</sup> Indeed, it was demonstrated that suppression of melatonin secretion by white light (at the intensity used to treat hyperbilirubinemia in human infants) in newborn rats or synthesis in adult rats (by administration of the beta-adrenoceptor blocker propranolol) lowered serum calcium concentration.<sup>150</sup> Moreover, in both studies, treatment of rats with melatonin prevented serum calcium decrease.<sup>150–152</sup>

The *in vitro* effect of melatonin on cellular proliferation and differentiation has stimulated interest in its role in bone regeneration. Therefore, the effect of melatonin on bone metabolism was recently examined using different kinds of osteoblastic cell lines.<sup>153,154</sup> Roth et al.,<sup>26</sup> for instance, examined the direct effect of melatonin on osteoblasts using MC3T3-E1 preosteoblasts and rat osteoblast-like osteosarcoma 17/2.8 cells.<sup>142</sup> Both cell lines in the presence of nanomolar concentrations of melatonin augmented gene expression of bone sialoprotein (an extracellular bone matrix protein that is expressed during osteoblastic cell differentiation and is required for mineralization), as well as several other essential bone marker proteins including alkaline phosphatase, osteocalcin, and osteopontin, and stimulated both osteoblast differentiation and mineralization.<sup>142</sup> This relationship is supported by the fact that the genes of a large portion of bone matrix contain the sequence of bases (RGGTCA) necessary for the nuclear receptor of melatonin to bind with its promoting zone.<sup>155</sup> In these preosteoblastic cell lines, melatonin seems to reduce the period of differentiation into osteoblasts, and this reaction seems to be mediated by the membrane receptors for the indole.<sup>156</sup>

Previous studies have shown that melatonin stimulates the synthesis and proliferation of collagen type I fibers in human osteoblasts *in vitro*.<sup>153</sup> Similar results were reported in clinically relevant human bone cells, in which micromolar concentrations of melatonin significantly increase procollagen type I<sub>c</sub> peptide production (a measure of type I collagen synthesis) in a concentration-dependent manner.<sup>153</sup> Some authors reported that the mitogen-activated protein kinase (MAP-K) signal transduction pathway may be responsible for melatonin's effects on osteoblasts differentiation,<sup>157–159</sup> even if further studies are needed. In another study, melatonin acted directly on human bone cells (HOB-M) and human osteoblastic cell line (SV-HFO) and dose-dependently increased the proliferation in both cell types by twofolds.<sup>153</sup> Type I collagen synthesis was also elevated in both cell types, but neither alkaline phosphatase activity nor osteocalcin secretion was influenced by melatonin.<sup>153</sup>

Furthermore, seems that these effects on osteoblasts are mediated through melatonin transmembrane receptors.<sup>160</sup> Two genes have been isolated for membrane melatonin receptors in mammals including humans: one is the melatonin 1a receptor<sup>161</sup> and the other is the 1b receptor.<sup>162</sup> In a recent study, reverse transcription–polymerase chain reaction and Western blot analysis showed that human osteoblasts express the melatonin 1a receptor and that its expression levels decrease gradually with age.<sup>163</sup> In this study, Satomura et al. confirm a possible role of melatonin in human bone formation, showing that at pharmacological doses, it is able to enhance proliferation and differentiation of normal human osteoblasts, even if its mechanisms of action remain unclear. Moreover, to demonstrate the possible utility of melatonin as a pharmaceutical agent to shorten the period of bone regeneration, the effects of this hormone on bone formation *in vivo* were also tested; in mice, intraperitoneally administered melatonin to mice induced a significant increase in the ratio of new to old bone mass in the cortex of the femur.<sup>162</sup> Collectively, all these findings indicate that melatonin has a promotional action on osteoblasts.

The bone complex, therefore, includes osteoblasts, osteoclasts, and the bone matrix. An interaction between osteoclasts and osteoblasts has been recently noted in mammals, and it is necessary to consider both their actions.<sup>146,164</sup> On the whole, osteoclasts are under the control of local modulator factors produced, among other cells, by the osteoblasts. The receptor activator of nuclear factor  $\kappa$ B (RANK) and the receptor activator of the nuclear factor  $\kappa$ B ligand (RANKL) have been identified in osteoclasts and osteoblasts, respectively.<sup>165</sup> Exposure of osteoblasts to substances such as parathyroid hormone stimulated the expression of osteoclast differentiating factors: in particular, it was found that the bound RANKL to RANK induces multinucleated osteoclasts (active type of osteoclasts)<sup>165</sup> and then can activate bone resorption.<sup>166</sup> Another osteoblastic protein, osteoprotegerin (a soluble member of the superfamily of tumor necrosis factor receptors), on the contrary, can inhibit the differentiation of osteoclasts by binding to osteoclast differentiation factor as a decoy.<sup>166</sup> The effect of melatonin on the expression of RANK and osteoprotegerin was investigated in mouse MC3T3-E1 osteoblastic cells.<sup>154</sup> In this study, melatonin at pharmacological doses causes an inhibition of bone resorption and an increase in bone mass by down-regulating RANK-mediated osteoclast formation and activation:<sup>154</sup> the authors observed a significant dose-dependent decrease of RANK mRNA and an increase in both mRNA and protein levels of osteoprotegerin in MC3T3-E1 cells. On the other hand, melatonin is capable of influencing the RANKL system, suppressing its activity<sup>36,154</sup> and favoring the formation of new bone: this indicates that melatonin may bring about a reduction in bone resorption and an increase in bone mass because of its repression of osteoclast activation by means of RANK.<sup>167</sup> Moreover, in *in vivo* studies on intact mice, pharmacological doses of melatonin elevated the bone mineral density<sup>154</sup> aside from the trabecular thickness of the vertebra and the cortical thickness of the femur already showed in ovariectomized mice.<sup>168</sup> This treatment significantly reduced the bone resorption parameters (osteoclastic surface and osteoclastic number) but did not increase the histomorphometric bone formation parameters (bone formation rate, mineral apposition rate, and osteoid volume).<sup>154</sup> So the skeletal effects of melatonin are, presumably, a result of the inhibition of osteoclast activity.

In a recent study, the effects of melatonin on osteoclastic and osteoblastic cells were examined using a culture system of the teleost scale.<sup>169</sup> The teleost scale is a calcified tissue that contains osteoclasts, osteoblasts,<sup>170</sup> and also components of the bone matrix;<sup>171,172</sup> hydroxyapatite also exists in the scale.<sup>173</sup> The scales of teleosts contain as much as 20% of the total body calcium and are a functional internal calcium reservoir during periods of increased calcium demand.<sup>169,174,175</sup> Thus, there are many similarities between the teleost scale and mammalian membrane bone. In this “*in vitro* assay system,” melatonin directly suppressed both tartrate-resistant acid phosphatase and alkaline phosphatase activities, markers of osteoclastic and osteoblastic activity, respectively, by suppressing their growth and differentiation.<sup>169</sup> This was the first report related to the function of melatonin in osteoclasts and on the inhibitory effect of melatonin in osteoblasts when incubated in the presence of osteoclastic cells. Indeed, the authors argued that the previously reported effects of melatonin to stimulate proliferation of mammalian osteoblasts<sup>142</sup> were artifacts because the experiments were

conducted with isolated osteoblasts, while in bone formation and metabolism, cell-to-cell contacts between osteoblasts and osteoclasts occur.<sup>146</sup>

Moreover, melatonin acts directly on osteoclasts, which use a variety of chemical agents and different mechanisms to resorb the extracellular matrix and degrade bone, including the production of free radicals. Osteoclasts generate high levels of free radicals, superoxide anions, in particular, during bone resorption, which contribute to the degradative process.<sup>176</sup> Thus, melatonin, being an antioxidant and a free radical scavenger at both physiological and pharmacological concentrations<sup>177</sup> may interfere in this function of the osteoclast detoxifying free radicals, which are produced during osteoclastogenesis,<sup>178</sup> leading to an inhibition of reabsorption of the bone.<sup>167</sup> Therefore, the effect of melatonin in preventing osteoclast activity in the bone may depend in part on its free radical scavenging properties. These data point toward an osteogenic effect of melatonin, which may be of clinical importance because it could be used as a therapeutic agent in situations when bone formation would be advantageous, such as in occlusal reconstruction using dental implants.

#### 13.4.1 MELATONIN PROMOTES BONE REGENERATION AROUND DENTAL IMPLANTS

All the actions of melatonin on bone metabolism described above are of interest, as it may be possible to apply melatonin during endo-osseous dental implant surgery as biomimetic agent.<sup>179</sup> Occlusal reconstruction using dental implants is of importance from the point of view of the quality of life of patients: for this reason, osseointegration should be promptly completed and it should be maintained for as long as possible. So, in order to obtain functional bone as soon as possible, it is critical to enhance at the same time both the proliferation and differentiation of osteogenic cells.

The long-term success of many dental implants depends on their ability to become well integrated in bone. Titanium (Ti) is the implant material of choice for use in dental applications, even if the surface properties of this material are not well suited for bonding to bone. Modifications of both surface topography and chemistry have led to significant improvements in the integration of such materials in bone. Several measures have been proposed to improve and accelerate osseous healing using topical treatments. They include the application of platelet-rich plasma, bone morphogenetic proteins, and growth factors (e.g., melatonin).<sup>180</sup> Tacheki et al. focused on the possibility that melatonin may be an effective hormone in the treatment of bone changes around dental implants; its efficiency has been shown when melatonin acts synergistically with fibroblast growth factor 2 (FGF-2) to promote bone formation around titanium implants placed in tibia of rat by enhancing both the proliferation and differentiation of osteogenic cells.<sup>181</sup> The results of Tacheki et al. strongly suggest that these two molecules have the potential to promote osteointegration of titanium implants, even if their exact roles during osteogenesis are not completely understood; FGF-2 is typically thought to control osteoprogenitor cell proliferation, whereas melatonin is more important in osteoblast differentiation. Moreover, in a recent study, it has been stated that topical application of melatonin successfully activated osteogenesis around titanium implants in a canine mandibular model. Considering a possible future application of melatonin in dentistry, the authors of this study thought that it was beneficial to introduce the effects of melatonin in implant treatment and showed that when applied topically, melatonin promotes peri-implant bone formation. A study with experimental beagle dogs was carried out to evaluate the effect of the topical application of melatonin mixed with a very biocompatible collagenized bone substitutes of porcine origin<sup>182,183</sup> on the osteointegration of dental implants. Bone grafts have been usually placed in bone defects or into extraction sockets to facilitate healing, used, for instance, in order to increase the width of the crest or for augmentation of the maxillary sinus floor to enable implant placement.<sup>184</sup> The results of Calvo-Guirado et al. showed that melatonin, combined with collagenized porcine bone substitutes, reveals more bone-to-implant contact and less crestal bone resorption than control implants, suggesting a positive role of melatonin in osteointegration around dental implants.<sup>185</sup>

As emerged from all these studies, melatonin, with its capacity to induce bone cell proliferation and differentiation, could facilitate the process of healing of bone tissue in dental implant surgery,

reducing the period of osteointegration and settling of the implant, and therefore, the quality of life of the patient may be improved.

### 13.5 MELATONIN USE IN SURGERY AS AN ANESTHETIC ADJUVANT

#### 13.5.1 POTENTIAL ANESTHETIC EFFECTS OF EXOGENOUSLY ADMINISTERED MELATONIN

General anesthesia is a pharmacologically induced state that entails amnesia, analgesia, hypnosis (unconsciousness), immobility, and blunted autonomic responsiveness.<sup>186</sup> Experimental literature in animals<sup>187</sup> and anecdotal observations in humans<sup>188</sup> have shown that melatonin has hypnotic actions.<sup>189</sup> Anton-Tay et al.<sup>190</sup> were the first to demonstrate clearly that exogenously administered melatonin has hypnotic properties in human subjects and that the loss of consciousness is accompanied by a pattern of electroencephalographic activity similar to that seen during intravenous and volatile anesthesia.<sup>191</sup> At the molecular level, general anesthetics enhance the function of inhibitory gamma-aminobutyric acid type A (GABA<sub>A</sub>): positive modulation of GABA<sub>A</sub> receptor function has been recognized as an important component of the central depressant effects of many intravenous anesthetics, including propofol.<sup>192</sup> There is evidence to suggest that the central effects of melatonin, at least in part, involve facilitation of GABA-ergic transmission by modulating the GABA receptor.<sup>193,194</sup> Also, significant dose-dependent increases in GABA concentrations were noted in the central nervous system after the administration of melatonin.<sup>195</sup>

In rats, intravenous administration of large doses of melatonin induced a profound dose-dependent hypnotic state that was characterized by a rapid loss of righting reflex and antinociceptive effects against thermal, chemical, and electrical stimuli (resulted from the release of b-endorphin<sup>196</sup>), less potent, but similar to that observed with equipotent doses of thiopental and propofol;<sup>197</sup> moreover, orally administered melatonin has been shown to potentiate the anesthetic effects of thiopental and ketamine.<sup>198</sup> Furthermore, in a study using melatonin pretreatment associated with thiopental (a hyperalgesic molecule), the latter did not increase paw withdrawal threshold;<sup>199</sup> these findings underlie the anesthetic adjuvant properties of melatonin. However, even if melatonin exhibited antinociceptive effects, it was not as effective as other anesthetic agents in abolishing the response to tail clamping. Loss of righting reflex (hypnosis) and abolition of purposeful movement response to tail clamp (immobilization) are used for determination of anesthetic potencies of volatile anesthetics.<sup>200</sup> Thus, melatonin on its own does not seem to possess sufficient efficacy to warrant consideration as a general anesthetic.

rephrased. OK?

The above observations raised the question whether melatonin might be suitable at least as an anesthetic adjuvant in preoperative treatments. Orally administered melatonin (5 mg) is used as a preoperative medication in both paediatric<sup>201</sup> and adult surgical patients.<sup>202,203</sup> The management of anxiety in children undergoing dental procedures, for instance, has developed considerably in the last decades. The approach with behavior management techniques combined with relative analgesia (inhaled nitrous oxide and oxygen) is unsuccessful in some children. In such cases, control of pain and anxiety poses a significant barrier to dental care, and sedation or general anesthesia is seen as the only option. Clinical trials with melatonin as premedication agent in anxious children under N<sub>2</sub>O/O<sub>2</sub> sedation for dental treatment have shown good results, but so far, only limited data are available.<sup>189</sup> Naguib et al.<sup>202,203</sup> noted that premedication with 0.05, 0.1, or 0.2 mg/kg sublingual/oral melatonin is associated with preoperative anxiolysis and sedation in adults and children without impairment of psychomotor skills or impact on the quality of recovery; oral melatonin is often used to enhance both the onset and quality of sleep for premedication of adult patients.<sup>204,205</sup> Furthermore, it should be mentioned that melatonin has been effective in preventing of postsurgical adhesions,<sup>206</sup> delirium,<sup>207</sup> and sleep disturbances.<sup>208</sup>

A number of melatonin analogues of greater potency and efficacy (e.g., 2-bromomelatonin, phenylmelatonin) are currently being studied to determine their effects on the induction of general anesthesia. In contrast to melatonin, 2-bromomelatonin was effective in abolishing the response to

a supramaximal stimulus, as tail clamping, even in some animals that did not lose their righting reflex. Substitution with a lipophilic substituent, bromine, at the 2-indole position of *N*-acetyl-5-methoxytryptamine increases the hypnotic and antinociceptive, as well as the melatonergic properties of this molecule.<sup>209</sup> The profile of the hypnotic properties of this melatonin analogue is similar to that induced by propofol, which has a rapid onset and a short duration of action, but unlike propofol, the reduced nocifensive behavior persisted for a longer period. Those data support the notion that 2-bromomelatonin might find use as an anesthetic agent.

### 13.5.2 EFFECT OF ANESTHESIA AND SURGERY ON MELATONIN HOMEOSTASIS

The effects of surgery and anesthesia on melatonin secretion and endocrine function have not been thoroughly evaluated, even if many factors, such as sleep disturbances, pain, drugs, and stress, associated with surgical procedures and anesthesia are all potentially capable of interaction with melatonin production.<sup>210,211</sup> A possible explanation for the change in melatonin and melatonin metabolites levels around surgery can be related to anesthesia, and in particular, the administration of benzodiazepines was found to partially suppress melatonin secretion.<sup>212</sup> It is also possible that the same influence is exercised by other anesthetic drugs. Reber et al.<sup>210</sup> reported that isoflurane and propofol anesthesia elicited elevated plasma melatonin levels. In contrast, Karkela et al.<sup>211</sup> reported that both spinal and general anesthesia significantly decreased melatonin secretion during the first postoperative period, noticing a postanesthesia phase delay in melatonin secretion. The conflicting results on melatonin secretion in the perioperative period in these studies could be due to the differences in the methodology of melatonin concentration measurement and in the variables of surgical procedures and preoperative preparation. Further studies are needed to better understand the short- and long-term changing levels of melatonin around surgical intervention.

## 13.6 CONCLUSIONS

From an accurate analysis of scientific literature, it seems that melatonin, either systemically or locally administered, has some interesting properties that both protect the oral cavity from inflammatory processes or infections and modulate the activity of cells involved in bone metabolism. Nowadays, experimental and clinical evidences are still inconsistent, so further studies are needed to clarify melatonin role in the homeostasis of oral tissues and enable the use of this hormone in the therapy of oral pathologies. Nevertheless, scientific community believes that assumptions exist to look at this molecule with attention.

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