SHORT COMMUNICATION

Melatonin could Alleviate the Dysregulation of Metabolic Reprogramming in Periodontitis—Implications in Host Modulatory Therapy

Thodur M Balaji[1], Saranya Varadarajan[2], Debasish Bandyopadhyay[3], Raghunathan Jagannathan[4], CJ Venkatakrishnan[5], Ashok K Bhati[6], A Thirumal Raj[7], Swaminathan Rajendiran[8], Shankargouda Patil[9]

Abstract

Periodontitis is an infectious disease of the tooth-supporting apparatus characterized by connective tissue breakdown and alveolar bone resorption ultimately resulting in tooth loss. A chronic immune response and jeopardized oxidant–antioxidant balance are cardinal features in the pathobiology of periodontitis. The resident cells of the periodontium are known to undergo metabolic changes in the pathogenesis of periodontitis. The principal cellular fraction of the periodontal ligament space in states of health, disease, repair, and regeneration is the periodontal ligament stem cells (PDLSCs). Although these cells are believed to adapt well to bacterial infections, a recent *in vitro* study has shed light on the metabolic changes in these stem cells infected with *Porphyromonas gingivalis* lipopolysaccharide. The findings of the study demonstrated elevated levels of Krebs cycle enzymes, succinate, and hypoxia-inducible factor 1 alpha (HIF-alpha) in the stem cells following *P. gingivalis* infection. In this context, we hypothesize a potential role that could be played by melatonin, an indoleamine molecule that has been found to play a significant role in periodontal homeostasis. It has been proposed that exogeneous melatonin supplementation in periodontitis could help in targeting metabolic dysregulation as melatonin is endowed with potent anti-inflammatory and antioxidant properties. Melatonin could also help in decreasing succinate production in the PDLSC by increasing alpha-ketoglutarate generation and could inhibit stabilization of HIF-alpha. Melatonin-mediated conversion of proinflammatory M1 macrophage to anti-inflammatory M2 macrophage phenotype could help in the resolution of periodontal disease and foster healing mechanisms in the diseased periodontium.

Keywords: Hypothesis, Melatonin, Metabolic dysregulation, Periodontitis.

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Introduction

The human periodontium is a composite structure that forms the tooth-supporting attachment apparatus. This complex tissue composed of the avascular cementum, fibrous periodontal ligament, and alveolar bone is cased in the gingiva (gums) which is a soft tissue forming an integral part of the oral mucosa.[1] Accumulation of dental plaque, a soft microbial biofilm around the gingival margins causes inflammation of the gingival tissues denoted as gingivitis.[2] Untreated gingivitis in some but not all cases extends to involve the periodontal attachment apparatus consequently leading to an irreversible condition called periodontitis. Periodontitis is clinically characterized by the formation of gum pockets that bleed upon probing. In some cases, this condition is associated with the formation of periodontal abscesses and tooth mobility which consequently leads to tooth loss.[3] Periodontitis is a disease with significant systemic impact as it has been proposed as a risk factor for cardiovascular disease,[4] diabetes mellitus type 2,[5] and adverse pregnancy outcomes.[6]

Regarding the pathogenesis of periodontitis, it has been understood that microbial challenge from the subgingival dental plaque biofilm[7] elicits a chronic inflammatory and immune response in the host periodontal tissues[8,9] coupled with an overzealous production of reactive oxygen species (ROS).[10] The component cells of the periodontium like the epithelial cells, fibroblasts, macrophages, bone cells, and periodontal ligament cells are believed to undergo metabolic changes in the pathogenesis of periodontitis due to a dysregulated immune and redox response as abovementioned. This phenomenon of metabolic reprogramming

[1]Department of Dentistry, Bharathirajaa Hospital and Research Institute, Chennai, Tamil Nadu, India

[2,7]Department of Oral Pathology and Microbiology, Sri Venkateswara Dental College and Hospital, Chennai, Tamil Nadu, India

[3]Department of Physiology, Oxidative Stress and Free Radical Biology Laboratory, University of Calcutta, University College of Science and Technology, Kolkata, West Bengal, India

[4]Department of Periodontics, Tagore Dental College and Hospital, Chennai, Tamil Nadu, India

[5]Department of Prosthodontics, Tagore Dental College and Hospital, Chennai, Tamil Nadu, India

[6]Division of Periodontics, College of Dentistry, Jazan University, Jazan, Kingdom of Saudi Arabia

[8]Department of Periodontics, Sri Venkateswara Dental College and Hospital, Chennai, Tamil Nadu, India

[9]Department of Maxillofacial Surgery and Diagnostic Sciences, Division of Oral Pathology, College of Dentistry, Jazan University, Jazan, Kingdom of Saudi Arabia

Corresponding Author: Shankargouda Patil, Department of Maxillofacial Surgery and Diagnostic Sciences, Division of Oral Pathology, College of Dentistry, Jazan University, Jazan, Kingdom of Saudi Arabia, Phone: +966507633755, e-mail: dr.ravipatil@gmail.com

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at the tissue level has been found to occur in many systemic conditions.[11] But a recently performed study on periodontal ligament stem cells (PDLSCs) in the periodontal ligament found significant changes.[12] Periodontal ligament stem cells are a mesenchymal stem cell population that forms a principal cellular fraction of the periodontal ligament space in states of health, disease, repair, and regeneration. These cells form and stabilize extracellular matrix components and due to their stemness and plastic properties, are believed to adapt well to bacterial infections.[13] Periodontal ligament stem cells are equipped with a wide array of pattern recognition receptors (PRRs), such as toll-like receptors (TLRs) and CD36, to sense the invading microbials, undergo fundamental changes in cell biology, and transmit danger signals to defense cells.[14] The interaction of PDLSC with macrophages in the periodontal ligament space and their influence on macrophage polarization from M1 (proinflammatory phenotype) to M2 (anti-inflammatory phenotype) subtypes has been previously documented.[15] In a recently performed *in vitro* study, it was found that infection of human PDLSC with *Porphyromonas gingivalis* lipopolysaccharide caused significant molecular and biochemical changes.[12] *Porphyromonas gingivalis* infection led to a shift in metabolic reprogramming from mitochondrial oxidative phosphorylation to cytosolic glycolysis in the PDLSC. This aberrant change has been termed by researchers as the Warburg effect which is classically defined as a phenomenon where pyruvate is used by cells as a metabolic substrate to release quick energy by aerobic glycolysis mechanism.[16] The Warburg effect has been documented to occur typically in cancer cells to acquire quick energy to undergo uncontrolled proliferation.[17] In the periodontal ligament cells also, an aberrant metabolic dysregulation was reported by the abovementioned study following bacterial infection. Profiling of the enzymes revealed that *P. gingivalis* infection of the PDLSC caused increased expression of the Krebs cycle enzymes isocitrate dehydrogenase, succinic dehydrogenase, and citrate synthase. It was found that the infected PDLSC accumulated high levels of succinate. The source of succinate in the PDLSC was contemplated to be derived through glutamine-dependent anaplerosis and the γ-aminobutyric acid (GABA) shunt although not demonstrated.[12] The findings of increased succinate dehydrogenase in the periodontal context are different from other *in vitro* models. In monocytes infected with LPS, it has been found that succinate elevation was coupled with dampened succinic dehydrogenase levels,[18] whereas *P. gingivalis* infection of PDLSC led to upregulated succinic dehydrogenase. The consequence of succinate elevation in the PDLSC was its export into the cytosol from the mitochondria which caused stabilization of hypoxia-inducible factor 1 alpha (HIF-alpha).[12] This situation in the PDLSC was found to create a pseudohypoxia-like situation and was found to turn on the transcription of proinflammatory cytokine genes. It was also observed that *P. gingivalis* infection of PDLSC caused a significant elevation of intracellular ROS.[12] The abovementioned novel experiment has pointed out some intricate metabolic changes at the cellular and molecular levels in periodontal disease. We hypothesize that melatonin administered exogenously could mitigate the deleterious effects of metabolic reprogramming that occurs in the periodontal tissues through multiple mechanisms. It is well known that endogenous melatonin plays a significant role in the pathobiology of periodontal disease. Melatonin also termed *N*-acetyl-5 methoxytryptamine is a product of tryptophan metabolism that is synthesized predominantly by the pinealocytes of the pineal gland.[19] It has been demonstrated that tissues other than the pineal gland could also synthesize and release melatonin. In the oral cavity, the salivary glands[20] and the gingiva[21] are endowed with the capacity of melatonin synthesis and also bear receptors for melatonin.[21] Melatonin receptors have been found on the gingival epithelial cells, gingival fibroblasts, and immune and inflammatory cells in the gingival connective tissues.[21] Melatonin has been found to perform numerous homeostatic functions in the human body. However, its depletion in periodontal disease necessitates exogenous supplementation which would exert profound effects as described below.

The Hypothesis

Melatonin administration could exert the following beneficial cellular and molecular effects in the periodontium. It is to be reiterated that it is not known if melatonin receptors are present on PDLSC. But the hypothesis still holds good based on the fact that melatonin exerts both receptor-independent and receptor-dependent effects. Hence, the below-mentioned effects of melatonin could be achieved in the PDLSC context even if melatonin receptors are not present on them.

- Decreasing succinate production by increasing alpha-ketoglutarate generation in the PDLSC followed by exosomal extrusion of the alpha-ketoglutarate into the periodontal microenvironment.
- Reducing the effects of succinate-mediated HIF-alpha stabilization in the PDLSC by dephosphorylation of p70S6K and its direct target RP-S6 by repressing the mammalian target of mTORC1.
- Potentially reducing the levels of ROS and NF kappa B in the PDLSC through antioxidant and anti-inflammatory mechanisms.
- Contributing to the conversion of the proinflammatory M1 macrophage phenotype to the anti-inflammatory M2 macrophage phenotype through the utilization of the alpha-ketoglutarate exosomes produced by PDLSC.

Melatonin and Its Association with Succinate and Alpha-ketoglutarate Production

Melatonin is a potent inhibitor of the Warburg effect which operates in cancer cells causing uncontrolled proliferation.[22] About elevated succinate, in PDLSC, the phenomenon was pointed out to occur due to glutamine-dependent anaplerosis and GABA shunt akin to macrophages challenged with LPS. At this point, the role of melatonin can be clearly explained. Melatonin has been found to increase glutaminolysis, thereby converting glutamine to glutamate in the adipose tissue-derived macrophage model.[23] The generated glutamate is further transported from the cytosol into the mitochondria to become alpha-ketoglutarate. The generated alpha-ketoglutarate under the influence of melatonin is packed into exosomes and extruded by the cells into the surrounding microenvironment. By this mechanism of alpha-ketoglutarate utilization, succinate generation could be countered by melatonin and its accumulation in the cytosol is also prevented. The exosomal extrusion of alpha-ketoglutarate has been demonstrated to occur in adipose tissue under the influence of melatonin where the adipocyte-derived alpha-ketoglutarate has been extruded into the microenvironment as exosomes.[24]

Effects of Melatonin on Succinate-mediated HIF-alpha Stabilization

As described earlier, the elevated succinate in the PDLSC consequently causes HIF-alpha stabilization and proinflammatory gene transcription. This mechanism could also be repressed by melatonin through dephosphorylation of p70S6K and its direct target RP-S6 by repressing the mammalian target of mTORC1.[23] By this described pathway, melatonin destabilizes HIF-alpha in the macrophage model. In the same method, melatonin could destabilize HIF-alphas in the PDLSC, thereby reversing the pseudohypoxic situation and downregulating HIF-alpha mediated proinflammatory gene transcription.

Influence of Melatonin on Intracellular ROS Production and NF Kappa B Activity

The *P. gingivalis* challenge was also found to increase intracellular ROS levels in the PDLSC. This reaction can be efficiently countered by melatonin as it is a powerful antioxidant under *in vitro* and *in vivo* conditions.[25] Several studies have been performed to demonstrate the antioxidant potential of melatonin. It has been shown that melatonin is superior to conventional antioxidants as one molecule of melatonin could scavenge up to ten ROS molecules.[26] Melatonin and its metabolites AMK and AFMK are endowed with the capacity of scavenging the most lethal hydroxyl radical implicated in many pathological conditions.[27] About NF kappa B, melatonin has been found as a potent inhibitor of this transcription factor.[28] An experiment on melatonin treatment found a reduction in NF kappa B production in *P. gingivalis* fimbriae-treated monocytes showing the anti-inflammatory effects of melatonin.[29] In addition to its antioxidant and anti-inflammatory effects, melatonin is also a cytoprotective agent.[30] Hence, it is expected that melatonin administration could exert antioxidant, anti-inflammatory, and cytoprotective effects on the PDLSC.

Effects of Melatonin on Macrophage Polarization

The exosomal transport of alpha-ketoglutarate from the PDLSC as previously described could further be taken up by macrophages in the periodontal microenvironment resulting in a polarization of macrophage phenotype from destructive M1 to a reparative M2 phenotype.[23] This kind of mechanism has been demonstrated under the influence of melatonin in adipose tissue where melatonin inhibits adipose inflammation through alpha-ketoglutarate.[24] There is enough body of evidence available to demonstrate the interaction and cross-talk between macrophages and PDLSC in states of periodontal health and disease.[31] Hence, this cross-talk could be efficiently modulated by melatonin administration which could favor a polarization of the macrophages toward the M2 phenotype with anti-inflammatory properties. Figure 1A summarizes the mechanisms of periodontal destruction, while Figure 1B summarizes how these mechanisms could be countered with an exogenous supplementation of melatonin.

Testing the Hypothesis

It would be worthwhile testing the positive influence of melatonin on PDLSC infected with *P. gingivalis* in an *in vitro* model. Since it is not known if PDLSC bears melatonin receptors, it would be worthwhile to assess if melatonin receptors are present in these cells by immunohistochemical techniques. Succinate, alpha-ketoglutarate, and HIF-alpha levels should be tested in PDLSC-infected cultures at baseline and following melatonin treatment to assess if melatonin exerts positive effects on metabolic reprogramming. Animal studies could be conducted to assess if melatonin treatment in a model of ligature-induced periodontitis could improve cross-talk between PDLSC and macrophages favoring a polarization from M1 to the M2 phenotype. If *in vitro* and animal studies yield positive results, human clinical studies in this direction can be performed.

Clinical Implications

Taking all the available evidence into account, melatonin could be used as an efficient host modulatory agent in periodontal therapy. It would be worthwhile to assess the efficacy of melatonin in the systemic formulation and topical formulations such as mouthwashes, gels, gummies, and toothpastes in controlling metabolic reprogramming in the periodontal tissues. It is previously known that the oral tissues bear melatonin receptors. Hence, melatonin could exert both receptor-independent and receptor-mediated effects in the management of the periodontal disease.

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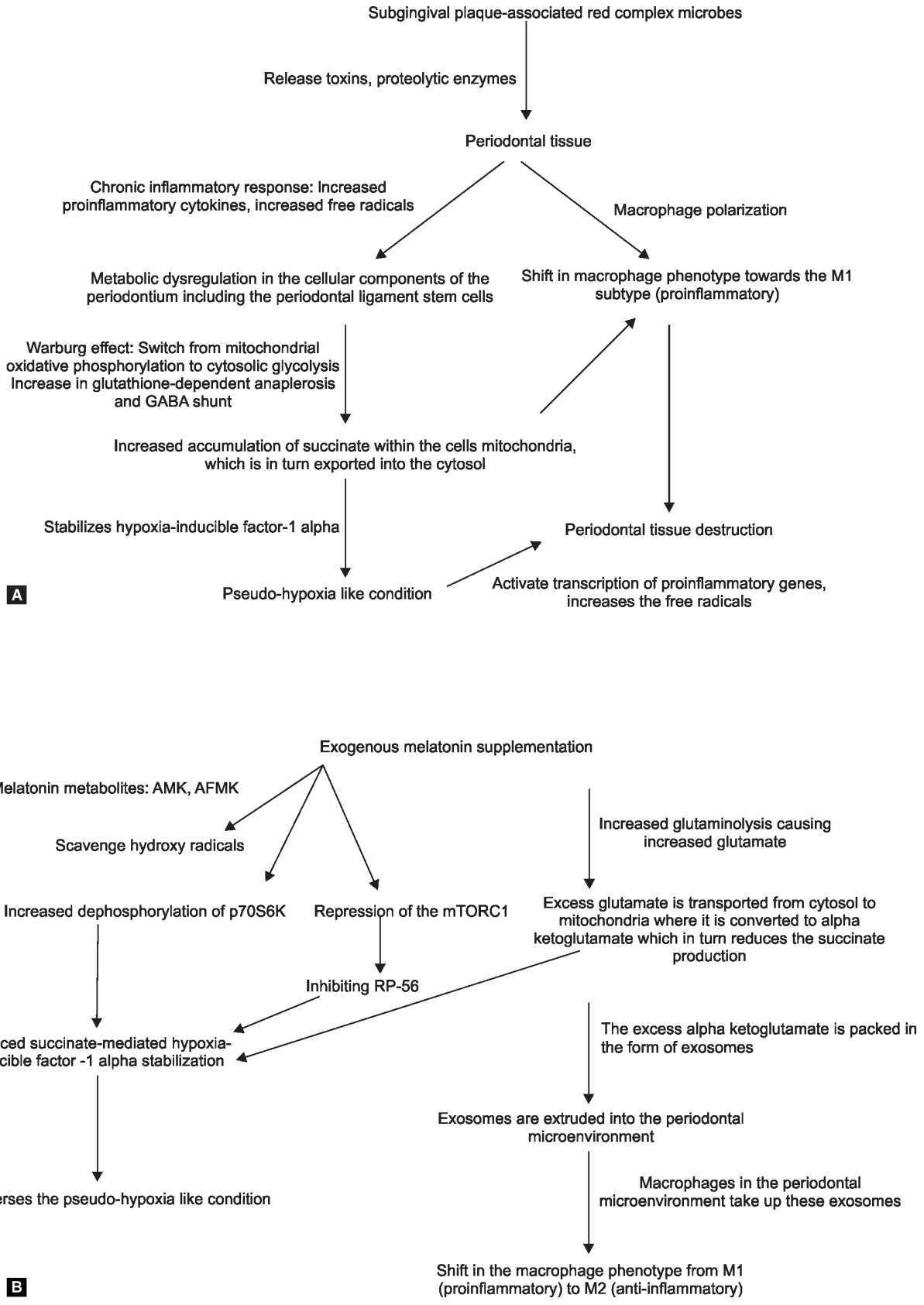

Figs 1A and B: (A) Summary of the mechanisms resulting in periodontal destruction; (B) Summary of the probable mechanisms through which melatonin could potentially alleviate tissue destruction in periodontitis

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