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The Photomodulation Activity of Metformin Against Oral Microbiome

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Abstract

Periodontitis is one of the most common inflammatory diseases of the periodontium, which results in the inflammatory destruction of supporting structures around teeth and is closely associated with the development of systemic disease. Due to a wide variety of antibiotic resistance periodontopathic bacteria, photodynamic therapy (PDT) is a non-invasive adjunctive therapeutic modality that is capable of destroying the whole range of microbes. Metformin (Metf) is an antidiabetic drug, and recent studies suggest that cancer patients who receive Metf and are exposed to radiotherapy and chemotherapy show better outcomes. Our surveys in this review introduce Metf as a potent stimulus in increasing the efficacy of PDT in the induction of destruction in microbial cells.

Keywords: Antibiotic resistance; Metformin; Periodontitis; Periodontopathic bacteria; Photodynamic Therapy;

Introduction

In the last decade, the number of antibacterial drugs approved with a new mechanism of action has declined.[1] The results of the misuse of these drugs are their adverse effects and particularly the expansion of bacterial resistance.[2] Resistance is often accompanied by the existence of antibiotic resistance genes which can be easily found in the oral microbiome.[3] The importance of the commensal microbiota lies in its function as a reservoir of antibiotic-resistant microorganisms that some of them are also able to create local and systemic diseases. It has been shown that antibiotic resistance associated with periodontal microbiota has increased.[4]

Tooth loss and alveolar bone resorption in individuals with periodontal disease have occurred.[5,6] In many countries, the incidence of periodontal disease has remained high, so that adults and young people are affected by severe periodontitis.[7] Famous pathogens in producing periodontal diseases include *Porphyromonas gingivalis* and *Fusobacterium nucleatum*.[8]

Several studies show that some diseases are more strongly associated with the presence of periodontitis such as diabetes mellitus,[9] rheumatoid arthritis,[10] bacterial pneumonia,[11] cardiovascular diseases,[12] adverse pregnancy outcomes[13] (premature birth, birth low birth weight, etc), and an increased risk of oral cancer.[14] Oral squamous cell carcinoma (OSCC) is common cancer worldwide but occurs more frequently in individuals with oral bacteria. Compared with normal mucosa, OSCC surfaces have higher levels of periodontal pathogenic bacteria.[15] In patients with untreated periodontal disease, they simplify the entry of bacteria and bacterial products into the bloodstream.[16] Oral diseases such as dental caries and periodontal disease are directly associated with biofilm-related infections of the oral cavity.[17] A biofilm is very resistant to antibiotics and human immunity.[18] Oral biofilm antibiotic resistance can be transferred into or out of the oral cavity.[19] the formation of the microbial biofilm is a common cause of morbidity and mortality in patients and leads to an increase in healthcare cost.[20]

Photodynamic therapy (PDT) has the potential to become established as an antimicrobial approach, which appears best for localized infections under the conditions where antibiotics are not effective in the treatment of infection.[21,22] Bacteria within the biofilms matrix are 2–1000 fold more resistant to an antimicrobial agent and PDT might be an optional therapeutic method to disrupt biofilms that cause oral disease via pathogenic bacteria.[23,24] Metformin (Metf) increases apoptotic response[25] and provides a synergistic advantage with chemotherapy

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and radiotherapy against certain cancers.[26,27] This review focuses on the evaluation of the effect of the combined treatment of Metf and PDT on the treatment of periodontitis. In this review, data on PDT, Metf and the effect of their combination (PDT+Metf) against microorganisms, were collected from the published articles in PubMed, Google Scholar, and Scopus databases and prepared a review of the mentioned subjects.

Photodynamic Therapy: Definition

PDT contains the components of the visible light, photosensitizer (PS) and oxygen[28] that form reactive oxygen species (ROS), causing the destruction of microorganisms.[29]

Mechanism of Action of Photodynamic Therapy

In the process of PDT, the components of the PS, when exposed to a particular light wavelength, gain a higher level of energy through the transition of electrons. In this exciting status, PS can react with oxygen and produce hydrogen peroxide (H_2O_2), superoxide anion radical ($O^{\cdot}_2$), and hydroxyl radicals ($^{\cdot}OH$) (process type I) or react with oxygen to initiate the formation of reactive singlet oxygen ($^1O_2$) (process type II).[30] The products produced in these reactions can cause considerable damage to the microorganisms or can change their metabolic activities irreversibly, thus resulting in death.[31] When compared to other therapeutic methods, PDT has multiple benefits. PDT is a non-invasive and successful method in the treatment of the periodontal infection which results in killing a large variety of pathogens.[32,33] The photo-activation allows better action for localized forms that decrease the complications of PDT, and can also be used to couple with other medical procedures. PDT is known as a cost-effective therapeutic approach due to the combination of low-cost PS and light sources. In addition, PDT has different cellular targets; thus, drug resistance does not happen.[34]

Metf: Definition

Metf (a biguanide drug) has commonly been used for decades for the treatment of diabetes type II. About 150 million people worldwide use Metf. It is derived from the plant Galega officinalis[35] and has recently been suggested as an adjuvant treatment for cancer.[36,37] Kim et al reported that radiation-sensitizing effect of Metf on hepatocellular carcinoma happened via increased apoptosis, cell cycle arrest, and enhanced DNA destruction.[38]

Metformin: Mechanism of Action

In eukaryotic cells, the primary target of Metf is complex-I of the electron transport chain leading to an accumulation of ROS and oxidative damage to lipids, protein, and DNA that could potentiate the effects of ionizing radiation.[39,40] Complex-I (proton-pumping NADH) is the first energy-transducing complex of many respiratory chains in eukaryotic cells and prepares the proton motive force required for energy consuming routes. In the respiratory chains of many bacteria, there is homologs of complex-I.[41-43]

In eukaryotic cells, the inhibition of complex-I by Metf results in decreased oxygen consumption and adenosine triphosphate (ATP) generation. Following the reduction in ATP production in eukaryotic cells, the cellular levels of adenosine monophosphate (AMP) increase and the energy sensor AMP-activated kinase (AMPK)[40] is activated. A serine/threonine protein kinase, known as AMPK, is comprised of a catalytic subunit (α) and two regulatory subunits (β and γ).[44]

When the amount of ATP concentrations is low, AMPK is activated and AMP concentrations enhance in response to food deprivation, hypoxia and Metf administration.[45] AMPK activation improves the potency of neutrophils or macrophages to kill bacteria. Phagocytosis of bacteria as part of an innate immune response in the presence of macrophages and neutrophils plays a crucial role in the control of inflammation.[46] In eukaryotic cells, various activators of AMPK have been shown to raise the phosphorylation of CLIP170 (CAP-Gly domain-containing linker protein 1), which are required for microtubule dynamics.[47,48]

Inhibitions of AMPK and expression of a non-phosphorylate CLIP-170 mutant result in the improved accumulation of CLIP-170 at microtubule tips and slower tubulin polymerization. Additionally, AMPK inhibition results in microtubule instability.[49] Pro-inflammatory responses and acute-phase proteins released during PDT can affect the immune system, which promote the penetration of a great number of inflammatory cells into the treatment site.

PDT immunological effects, when used for the treatment of local infections, make treatment more effective.[50,51] The results of studies have shown that 5-aminolevulinic acid (ALA)-mediated PDT effectively induces oxidative stress. ALA itself is not a PS and operates as the biological precursor in the heme biosynthetic pathway that can be biosynthesized in nearly all aerobic cells in mammals. Exogenous ALA administration leads to the accumulation of protoporphyrin IX in the mitochondria, which causes destruction to the mitochondria, reduces cellular ATP and causes impairment of mitochondrial function, following cell death after light irradiation.[52-54] Furthermore, ALA has been shown to have considerable photo bactericidal activity and ALA could induce photodynamic inactivation effectively against various types of bacteria.[52]

Nitric Oxide

Nitrate is a component of human saliva that is converted to nitrite and nitric oxide (NO) rapidly by oral bacteria.[55] NO is a ubiquitous, free radical gas, which plays a major role in various physiological and pathological processes[56] and may act as a potential biological marker

for the detection of generalized chronic periodontitis.[57] NO is produced via nitric oxide synthase (NOS) in the oral mucosa. The inducible type of NOS (iNOS) is one of the NOS isoforms, which is enhanced in the presence of periodontal disease.[58,59] The intervention of NO in bone loss processes[60] and the expression of iNOS in oral dysplasia and oral lichen planus have been reported. Also, DNA destruction caused by NO increases and may lead to the advance of oral cancer.[58,61] NO signals not only greater resistance to photokilling, but also a changed phenotype in surviving tumor cells which are characterized by more aggressive proliferation, migration, and invasion.[62] In tumor cells, oxidative stress and leukocyte recruitment are NO-sensitive processes, which are induced by PDT[63] and iNOS inhibitors in these cells can alleviate resistance to photokilling.[64]

Metf has inhibitory effects on the complex-I and therefore creates superoxide anion that reacts with NO to form reactive nitrogen species (RNS) such as peroxynitrite ($ONOO^-$), which is a very potent inducer of DNA damage.[65,66] Activation of AMPK is induced by ONOO[67] and would inhibit iNOS by reducing the transcription of iNOS.[68]

PDT induces the increase of superoxide dismutase activity. Excessive levels of superoxide during oxidative stress cause a reduction in NO by forming peroxynitrite.[69,70]

Metf, coupled with PDT, has a prooxidant role in the tumor cells and induces apoptosis in tumor tissue, compared to the administration of each one alone. NO levels reduce, thereby elevating nitrotyrosine formation. Nitrotyrosine is a stable indicator for RNS generation and confirms that they have a useful role in activating AMPK.[25] In tumor models identified by moderately high production of NO, lowering the NO levels after PDT by NOS inhibitors (administered intravenously) appears to increase the rate of damage to treated tumors, as proposed by the improved tumor cure rates.[71]

Apoptosis

Apoptosis is a normal physiologic route, playing the main role in periodontitis.[72,73] Disorders of apoptosis may contribute to a wide range of pathologies including oral diseases.[74] Bacterial products produce pro-inflammatory factors. The inflammatory response has an important role in the expansion and progression of periodontitis.[75]

IL-4 is considered an anti-inflammatory cytokine that can modulate macrophage function and induce apoptosis in macrophages and monocytes.[76] In patients with generalized aggressive periodontitis, the concentration of IL-4 is lower compared with healthy persons.[77] Lack of IL-4 inhibits apoptotic cell death and may be responsible for the accumulation of macrophages in the inflammatory lesion and hence may contribute to the chronicity of the disease.[78,79] Caspases, Tumor protein p53 (p53), and B-cell lymphoma 2 (Bcl-2) family members are chief factors in the apoptosis process (Figure 1).[80-82]

P53 Tumor Suppressor

The p53 tumor suppressor functions as a transcription factor which becomes activated by numerous stress stimuli.[83] Some bacterial pathogens also actively prevent p53 protein and induce its degradation, resulting in variation of cellular stress responses.[84] The infection of the gingival epithelial cell with *P. gingivalis* results in a decrease in p53 levels. By phosphorylation of kinases like Chk2, Aurora A, CK1delta and CK1epsilon, P53 is activated. All of these kinases are downregulated by *P. gingivalis*.[85] A role for lipopolysaccharide (LPS) in the dysregulation of p53 has been confirmed.[86] Dysregulation of immune pathways involved in periodontal disease causes chronic inflammation and tissue destruction.[87] It has recently been reported that there is a relationship between P53 and increased immune response.[88] Apoptosis can be induced via the expression of p53.[89] PDT improves *TP53* gene amplification. The major role of p53 in the PDT process

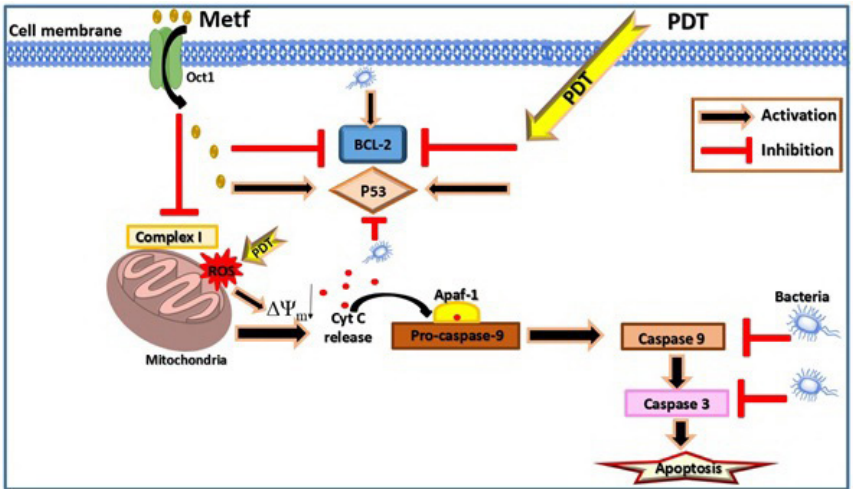

Figure 1. Interaction Between Metf, PDT and Microorganisms.

has been shown for numerous clinically approved PSs.[90,91] Metf induces radiosensitization in cancer cells lacking functional p53.[92] Metf activates AMPK, which induces phosphorylation of p53 on serine 15.[93,94] During cellular stress, phosphorylation at Ser-15 may be a serious event in the up-regulation and expression of p53.[95]

Therefore, providing maximal therapeutic advantages in combination with PDT, Metf can increase the activity of P53 and thus microbial cells are more sensitive to PDT and increased apoptosis.

BCL2 Family

BCL2, as an anti-apoptotic protein, can inhibit cell death induced by a range of stimuli and prevents the release of cytochrome c (Cyt *c*) from mitochondria.[96] The higher incidence of BCL2 expression occurs in patients with chronic periodontitis and generalized aggressive periodontitis, as compared to the healthy gingival tissues.[72,97] A significant tactic for *P. gingivalis* to survive in periodontal tissues is the ability to repress apoptosis.[98] *P. gingivalis* can block apoptosis in gingival epithelial cells by the up-regulation of the BCL2.[99] PDT degrades the BCL2 molecule, leading to apoptosis.[100] BCL2 may undergo direct oxidative damage from PDT-generated ROS.[101] BCL2 overexpression would protect cells from PDT-induced apoptosis and lead to impaired apoptosis after PDT.[102] BCL2 forms a main factor of photokilling and shows that when PDT is joined with the suppressive factor of BCL2 function, synergistic effects can occur.[103] *In vivo*, Metf decreases BCL2 expression, thereby inducing apoptosis.[104] Therefore, the use of Metf in combination with PDT can increase apoptosis and the efficacy of PDT in the treatment of bacterial infections.

Caspase Family

Caspases (a cluster of intracellular cysteine proteases) are capable of cleaving substrates after aspartic acid residues and create certain morphological shifts related to apoptosis.[105] Caspase-3 is regarded as a more important player in apoptosis and cell death.[106] Another important point in the induction of apoptosis is the stimulation of the apoptotic initiator caspase 9.[107] During *P. gingivalis* infection, activation of both caspase-9 and caspase-3 are blocked strangely.[15] Blocking the caspases results in a partial block in the loss of mitochondrial membrane potential ($\Delta\Psi_m$) and as a result, the diffusion of Cyt *c* is stopped.[108,109] The inhibition of caspase activation also blocks ROS production and promotes infection.[110,111] Due to the production of ROS within mitochondria, loss of $\Delta\Psi_m$ PDT causes mitochondrial damage.[112]

The prevention of complex-I and the generation of ROS induced by Metf should decrease the mitochondrial membrane potential[113,114] and apoptosis by releasing Cyt *c* from the mitochondrial inner membrane to the cytosol.[115] Cyt *c* by binding to proteins such as apoptotic protease activating factor 1 (Apaf 1) and procaspase 9 leads to the consecutive activation of caspase 9 and caspase 3, thus obligating the cell to apoptosis.[116] It can be concluded PDT combined with Metf increases ROS production, result in structural damage in microbial cells . Therefore, use of PDT in combination of Metf may increase antimicrobial efficacy of PDT against local infections.

Matrix Metalloproteinases Family

Matrix metalloproteinases (MMPs) are members of a multigene family of zinc-containing enzymes that are capable of degrading all extracellular matrix (ECM) components.[117,118]

Deep periodontal pockets form due to the damage to ECM and alveolar bone loss. There are diverse pathways for the metabolic destruction of ECM. One pathway seems to be due to the activation of MMPs[119] that may be involved in the degradation of collagen.[120] Type I collagen is the most commonly lost part of the periodontium.[121] MMP-2 has helicase activity that can cleave type I collagen.[122] MMP-2 level is increased during inflammatory conditions such as periodontal disease.[123] As we know, *P. gingivalis* is a key organism associated with the destruction of periodontal tissues.[124] *P. gingivalis* LPS can stimulate the production of prostaglandin (PG) E2 and promote the release of the MMP, which is associated with the development of gum disease.[125]

The enzyme cyclooxygenase-2 (COX-2) is responsible for the PGE2 production at sites of inflammation.[126] COX-2 is induced by bacterial LPS; commensal bacteria might regulate constitutive COX-2 expression.[127] Patients with periodontal disease have more COX-2 than healthy people.[128]

PDT induces MMPs, COX-2 expression, and release of PGE2, and the adjunctive use of MMP and COX-2 inhibitor enhances PDT responsiveness.[129-131] Metf has been reported to inhibit MMP-2 and COX-2 expression.[132] The inhibition of MMP2 ameliorates mitochondrial damage,[133] and the blockage of COX-2 expression increases the transcriptional activity of p53 and simplifies the decrease of $\Delta\Psi_m$ induced by PDT.[134]

Metf plus PDT can diminish MMP-2 activity and COX-2 expression in tumor tissues, compared to the patient who is treated with PDT. As a result of these effects, the combined use of inhibitors of COX-2 and MMP such as Metf as modulatory agents with aPDT with a reduction in $\Delta\Psi_m$ enhances the release of Cyt *c* and also increases the activity of P53. Moreover, it can induce apoptosis and prevent the tissue damage associated with periodontitis.

NF-κB Signaling

Nuclear factor kappa B (NF-κB) proteins are activated by microbial pathogens.[135] NF-κB has a fundamental role in the suppression of apoptosis induced by bacterial components like lipopolysaccharide.[136] Lipopolysaccharide, hypoxia or decreased oxygen availability can induce NF-κB.[137,138] *P. gingivalis* and

F. nucleatum activate the NF-κB pathway.[139] Toll-Like Receptors (TLRs) are strong activators of the NF-κB intracellular pathway.[140] Activation of TLRs on the cells of the periodontium leads to the overstated release of pro-inflammatory mediators, which may cause host tissue destruction.[141] TLR4 expression is blocked by the NF-κB inhibitor.[140] Neutralization of the NF-κB pathway might provide a useful therapeutic strategy in periodontitis.[142] PDT reduces inflammatory markers NF-kB and inflammatory cytokines.[143] Results show that TLR-4 level reduces significantly following PDT, which could have been due to damage/ inactivation of the LPS.[144]

Metf inhibits activation of NF-kB through the blockade of the phosphoinositide 3-kinase (PI3K)/Akt pathway.[145,146] The PI3K-Akt signaling pathway is of considerable importance for survival and apoptosis.[147] PI3K/Akt pathways are activated by TLR4 signaling via LPS.[148] PI3K/Akt activity is required for NF-kB activity.[149]

Conclusions

The use of non-specific drug Metf in combination with PDT can be a solution for enhancing the effectiveness of PDT, leading to its potential role in the reduction of periodontal infections. This combination therapy induces oxidative stress, increases the rate of apoptosis, reduces levels of NO, and decreases expressions of MMP-2 and COX-2. Furthermore, it causes the inactivation of the NF-κB signaling pathway to occur. The experimental data for determining the dose of Metf and getting a better response during clinical trials are required. There has been little research in this area and this study could open a new approach in the treatment of bacterial infections.

Ethical Considerations

Not applicable.

Conflict of Interests

The authors declare no conflict of interest.

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