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AN IN VITRO EVALUATION OF MELATONIN AS AN ANTIBACTERIAL AGENT ON PERIODONTAL PATHOGENS

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ABSTRACT

Aim and Objectives: Periodontal disease is an inflammatory condition of the periodontium caused by the imbalance in host bacterial interaction. Various agents have been used as biomarkers in the pathogenesis of periodontal disease. Melatonin, an indole amine hormone secreted by pineal gland is one such hormone. Melatonin has been declared an ubiquitous molecule, exerting antibacterial effect against various antibiotic resistant strains of microorganisms. However its effect against periodontal pathogens is not yet clear. Hence this in vitro qualitative analysis was conducted to evaluate the antibacterial efficacy of melatonin on selected periodontal pathogens.

KEY WORDS: Melatonin, Periodontal pathogens, Pineal gland, Minimum inhibitory concentration.

INTRODUCTION

Periodontitis is the inflammation of periodontium that extends beyond the gingiva and produces destruction of the connective tissue attachment of the teeth.[1] Various etiological factors have been associated with pathogenesis of periodontal disease, the most important being the microbiological etiology.[2,3] A wide array of micro organisms have been associated with periodontal disease, out of which *Aggregatibacter actinomycetemcomitans (Aa), Porphyromonas gingivalis (Pg), Prevotella intermedia (Pi)* and *Streptococcus mutans(S mutans)* have been predominantly associated with periodontal diseases.[4,5]

Despite advances in research methodology and laboratory assays in order to identify factors associated with chronic periodontal disease, it is still unclear how to potentially predict periodontal disease progression.[6] Hence various biomarkers are used to predict the periodontal disease progression. Melatonin is one such marker which has been associated with periodontal disease.[7]

Melatonin, an indole amine hormone secreted by the pineal gland has various actions in the oral cavity. Melatonin is not only present in human pineal gland but also in various taxa of organisms, different parts of plants, in invertebrates and vertebrates species. It acts as an antioxidant, immune modulator and promotes bone formation.[8] The levels of melatonin have been shown to be altered in periodontal disease indicating that it can be used as one of the biomarkers of periodontal disease progression.[7,9] There are multiple invitro studies on melatonin, only few studies stress on its antibacterial properties. Melatonin has also been shown to exert antibacterial activity against certain antibacterial resistant strains of gram positive and gram negative bacteria.[10] Some studies also suggest that melatonin has limited antibacterial properties.[11,12] However its antibacterial actions against various periodontal pathogens are yet to be evaluated. Considering the analogy of melatonin levels in periodontal health and disease, this qualitative in vitro study was aimed to assess the antibacterial efficacy of melatonin tablet(3mg) as a solution on isolated strains of aerobic and anerobic periodontal pathogens.

MATERIAL AND METHODS[10]

Preparation of melatonin solution[13]

Melatonin tablet (3mg) was dissolved in dimethyl sulfoxide solution (DMSO). 400µl of this solution was used to test the MIC.

Bacterial strains

MIC was tested for 2 isolated strains of aerobic organisms, *E.fecalis* (ATCC. No. 35550), *S. mutans*

(ATCC. No.25175) and 3 isolated strains of anerobic organisms *P.gingivalis* (ATCC.No.33277), *P.intermedia* (ATCC. No.25611), *A.actinomycetemcomitans* (ATCC.No.29523).

The minimum inhibitory concentration[13]

First the given organisms were grown in pure form. The minimum inhibitory concentration for melatonin was tested using microdilution/ serial dilution broth method. Four hundred microlitre of the prepared melatonin solution was taken in first tube. Two hundred microlitre of brain heart infusion (BHI) broth was added from 2nd tube to last tube. Two hundred microlitre of melatonin was diluted from 2nd to the last tube and then 200 µl of each organism was added in the tube. The tubes were incubated in anaerobic jar for 24 to 48 hrs and then checked for the turbidity.

The MIC was defined as the lowest concentration of melatonin that restricted growth. To authenticate the data the test was conducted thrice with the same concentration and same strains of microorganisms.

RESULTS

The results of the present study show that melatonin exerts antibacterial activity (Table: 1 and 2). All the organisms showed sensitivity to melatonin. Aerobic organisms, *E Fecalis* was sensitive at a minimum concentration of 0.375 µg/ml and *S mutans* at 0.0117 µg/ml (Table:1).

Table 1: MIC of melatonin against aerobic organisms.

Sr. No	Aerobic Organism	3.0 µg/ml	1.5 µg/ml	0.75 µg/ml	0.375 µg/ml	0.1875 µg /ml	0.0937 µg /ml	0.0468 µg /ml	0.0234 µg /ml	0.0117 µg /ml	0.0058 µg /ml
1.	***E.fecalis***	S	S	S	S	R	R	R	R	R	R
2.	***s. mutans***	S	S	S	S	S	S	S	S	S	R

Anaerobic organism *Pi* was sensitive at a concentration of 0.375 µg /ml, *Pg* at 0.1875 µg /ml and *Aa* at 0.0234 µg /ml respectively (Table: 2).

Table 2: MIC of melatonin against various anerobic pathogens.

Sr. No	Anaerobic Organism	3.0 µg/ml	1.5 µg/ml	0.75 µg/ml	0.375 *µg/ml*	0.1875 µg /ml	0.0937 µg /ml	0.0468 µg /ml	0.0234 µg /ml	0.0117 µg /ml	0.0058 µg /ml
1.	***Pi***	S	S	S	S	R	R	R	R	R	R
2.	***Pg***	S	S	S	S	S	R	R	R	R	R
3.	***Aa***	S	S	S	S	S	S	S	R	R	R

DISCUSSION

Periodontal disease is multifactorial however the main etiology being the microbial interaction with the host. The management of periodontal disease emphasizes on the disruption of this microbial insult either mechanically or chemically. Various antimicrobial agents like amoxicillin, metronidazole, tetracycline etc[14,15] have been used to treat periodontal disease either as a monotherapy or as an adjunct to mechanical therapy. However, the microbial resistance to various antimicrobial agents has become critical issue off late.[10,16] Therefore to counteract this issue, various plant extracts and other drugs are tested and tried for antibacterial efficacy. Melatonin an indole amine hormone is one such ubiquitous molecule which has been widely studied.

Melatonin has been shown to be immune modulatory, antioxidant, promotes bone formation.[8] Its role as a biomarker in periodontal disease has been studied.[7,9] Melatonin being a biomimitic molecule may have a potential role to counter the antibiotic resistance. Therefore this study was conducted to evaluate the antimicrobial efficacy of melatonin on various isolated strains of periodontal pathogens.

It has been studied that *Aa, Pg, Pi and S mutans* are the main aerobic and anaerobic organisms which are associated with periodontal disease.[17] Hence in this study, isolated strains of aerobic organisms like *E.fecalis* (ATCC. No. 35550), *S. mutans* (ATCC. No.25175) and 3 isolated strains of anaerobic organisms *P. gingivalis* (ATCC. No. 33277), *P. intermedia* (ATCC. No.25611), *A. actinomycetemcomitans* (ATCC.No.29523) were used to test the MIC of melatonin tablet. Serial dilution or microdilution broth method was used to check the MIC. This method is simple, accurate and reliable; therefore it is used to test the efficacy of any antimicrobial agent invitro.[18]

Melatonin is weakly soluble in water hence dimethyl sulfoxide (DMSO) was used to dissolve melatonin. Investigators have used ethanol to dissolve indoleamine but it is seen that at higher concentration melatonin precipitates with alcohol: water mixture,[10] therefore DMSO was used to dissolve melatonin. To authenticate the results, the study was conducted thrice using same method and same concentration.

The results of the present study showed that melatonin restricted the growth of periodontal pathogens [Table:1-2]. It was seen that aerobic organisms *E Fecalis*

was sensitive at a minimum concentration of 0.375 µg /ml and *S mutans* at 0.0117 µg /ml. Anaerobic organism *Pi* was sensitive at a concentration of 0.375 µg /ml, *Pg* at 0.1875 µg /ml and *Aa* at 0.0234 µg /ml respectively. Therefore confirming the antibacterial action of melatonin against periodontal pathogens. The results of the present study are in correlation with a study conducted by Tekbas FO et al.[10] Various explanations for the present results could be as follows. Firstly, besides being an ubiquitous, melatonin is a versatile molecule. Its ability to limit the growth of variety of tumour types confirm its versitality. Several mechanisms have been proposed to explain this. It has been shown that melatonin prevents the uptake of growth factors (fatty acid like linoleic acid) by the cancer cells thereby prevents the cell proliferation.[19] Analogous actions of melatonin on cell membrane of bacteria could be one of the reason for its antibacterial action.

Secondly, indole amine molecules have a metal binding capacity.[12] Bacteria are dependent on metals like iron for their growth and melatonin which crosses all the biological barriers, crosses the bacterial cell wall also thereby preventing the uptake of iron. This action leads to the reduction of bacterial cytoplasmic growth and therefore exerting antibacterial activity. It mainly affects the two phases of bacterial growth one is the lag phase and the log phase or the exponential phase. It reduces the absorption of substrates through the bacterial cell wall and binds to some of the intracellular substrate thereby leading to the prolongation of the lag phase of bacteria and inhibiting its exponential phase.[12] This in turn leads to the bacteria to enter death phase. In a study conducted by Gitto E et al it has been shown that melatonin is used to treatment of septic newborn also.[20]

The results also show that anerobic organism *Aa* was more sensitive than aerobic organism *S mutans* and *E fecalis* which is in accordance to the study conducted by Tekbas FO et al.[10] *S mutans* (0.0117 µg /ml) showed sensitivity at a lower concentration as compared to that of *E fecalis* (0.375 µg /ml). *Aa* (0.0234 µg /ml) and *Pg* (0.1875 µg /ml) showed sensitivity at a lower concentration as compared to *Pi* (0.375 µg /ml), demonstrating that melatonin may be used as an antibacterial agent. Melatonin resitricted the growth of *Aa* at a very low concentration, indicating that it could be a promising drug in the treatment of aggressive periodontitis.

With the emergence of antibiotic resistant strains of micro organisms and the risk of side effects of antimicrobials, melatonin has been shown to be effective against antibiotic resistant strains of micro organisms[10] and has relatively less side effects. Hence, it can be used as an effective anti microbial agent. However since this study is first of its kind, further in vivo studies have to be conducted to validate this hypothesis.

CONCLUSION

This study shows the antibacterial activity of melatonin against various potent periodontal pathogens at a very minimal concentration. The possible side effects of the drug at this concentration are negligible and therefore may be used to treat variety of bacterial infections. However to substantiate this data, further invivo study has to be conducted.

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