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## ORIGINAL ARTICLE

# Lethal photosensitization of oral pathogens via red-filtered halogen lamp

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**OBJECTIVE OF INVESTIGATION: The ability of laser irradiation in the presence of photosensitizing agent to induce lethal effect on oral bacteria is well documented. We designed an *in-vitro* experiment to achieve phototoxic results on two common oral pathogens, using a high intensity, red filtered halogen lamp. Our goal was to determine the minimum duration of light exposure and drug dilution to achieve at least 50% reduction in bacteria counts.**

**METHODS: Two common oral pathogens, *Porphyromonas gingivalis* and *Prevotella intermedia* were used in experiments. The source for light energy was a continuous working, high intensity, red filtered, halogen lamp (HL) with light transmitted through a flexible light guide over petri dishes containing live bacteria. Microorganisms were exposed to light for 5, 10 and 20 min. Methylene Blue (MB) in concentrations of 0.1, 0.075, 0.05, 0.025 and 0.01% was used as a photosensitizing agent. Light energy alone and MB alone was used as controls.**

**RESULTS AND DISCUSSION: Optimum lethal photosensitization (50% or more bacteria killing) of oral pathogens was achieved using halogen light illumination for 5 min and longer with 0.05% MB or exposure to light for 20 min in the presence of 0.025 and 0.01% MB. Light exposure of 20 min in the absence of MB was not effective in killing bacteria. In the absence of light, MB at concentrations of 0.025 and 0.001% was not effective. Reduction of bacteria with the use of 0.05% MB alone was also insignificant. However, 0.075 and 0.1% MB, even in the absence of light was found to be bacteriocidal.**

**CONCLUSIONS: Our *in-vitro* data indicate that we were able to achieve lethal photosensitization of two common oral pathogens with high intensity red filtered HL in the presence of diluted MB. In this era of increased incidence of antibiotic resistance, bacterial killing with laser or light energy in the presence of photosensitizing agent can prove to be a valuable treatment modality.**

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## Introduction

The oral cavity of humans is a host place to over 600 of bacterial species (Kazor *et al*, 2003). These bacteria form a unique ecosystem with complex interactions between species with ones producing substrates consumed by others. Some environmental changes, like diet, or decrease of host immune resistance may lead to the overgrowth of unfavorable species, known as periodontopathogens. The most common species included in this category are *Porphyromonas gingivalis*, *Prevotella intermedia*, *Fusobacterium nucleatum*, *Treponema denticola*, *Actinobacillus actinomycetemcomitans* and *Bacteroides spp*. The named bacteria are associated with chronic periodontitis, which is a major cause of tooth loss in adults when inhabiting the periodontal pockets (Loesche and Kazor, 2002). They are also believed to cause halitosis (or bad breath) when present on the back and base of the tongue (Wilson, 1994; Kazor *et al*, 2003). Traditionally, antibiotics are used to destroy offending bacteria in the cases of inflammatory conditions. Nevertheless, in chronic conditions, such as those caused by the named bacteria, there are many disadvantages in the long-term use of antimicrobial agents, both systemically and topically, notably the development of resistance rendering the agents clinically ineffective and difficulties arising from disturbance of the oral microflora and/or gastrointestinal disturbance. Many therapeutic regimens used for oral infections eliminate both pathogenic and commensal organisms indiscriminately, thereby disrupting the natural ecosystem of the oral cavity (Wilson, 1994).

Photodynamic therapy is a relatively new treatment modality, which was primarily used to destroy tumor cells with the power of laser light energy. Targeting the power to the pathologic cells was reached by staining them with specific dyes that are able to selectively absorb light energy of certain wavelength and are accumulated by tumor cells in lager quantities than by normal cells. The same principle, named lethal

photosensitization, using different dyes (photosensitizers) was later applied to killing different species of bacteria with a significant level of success. Lethal photosensitization is a process by which a photosensitizer is activated by light of an appropriate wavelength resulting in the production of cytotoxic oxygen free radical species, which then kill the target cell (Dougherty *et al*, 1998). Lethal photosensitization is not a specific modality and has been shown to be effective against a variety of cells such as those in neoplasms (Dougherty *et al*, 1990), fungi (Paardekooper *et al*, 1992, 1993, 1995; Wilson and Mia, 1994), viruses (Smetana *et al*, 1998), and bacteria (Bertoloni *et al*, 1990; Dobson and Wilson, 1992; Wilson, 1994; Nitzan *et al*, 1995; Merchat *et al*, 1996; Orenstein *et al*, 1997; Bhatti *et al*, 1998). Bacteria are killed as a result of membrane and DNA damage due mainly to the production of singlet oxygen on irradiation of the dye (Bhatti *et al*, 1998). Work carried out by Dobson and Wilson (1992) using Toluidine Blue O (TBO) as a photosensitizer in combination with HeNe laser light showed that, in dental plaque samples containing *P. gingivalis*, *Fusobacterium nucleatum*, streptococci, black-pigmented anaerobes, and *Actinobacillus actinomycetemcomitans*, all species were susceptible to lethal photosensitization. Furthermore, development of resistance to PDT would appear to be unlikely since its bactericidal activity is due to singlet oxygen and other reactive species such as hydroxyl radicals, which affect a range of cellular targets (Dobson and Wilson, 1992; Paardekooper *et al*, 1992, 1993; Berthiaume *et al*, 1994; Bhatti *et al*, 1998).

The fact that lethal photosensitization is not specific is advantageous in one respect; it is possible to kill all the bacteria present in a mixed infection. However, this also means that commensal bacteria and host tissues could be adversely affected, especially with high-density local power output of the laser light sources. This consideration as well as the others, like hazards related to the use of laser devices, has prevented broad clinical implementation of photodynamic therapy (PDT) in treating different oral conditions.

The goal of this study was to find alternative light source that could be used to apply this promising principle in fighting the unfavorable oral microbiota in broad clinical practice. We assumed that power density produced by high intensity red filtered halogen lamp may be sufficient to activate certain dyes and cause sufficient bacterial killing without serious damage to commensal bacteria or oral mucous lining. This could also eliminate or decrease the other potential hazards associated with the use of laser devises, such as eye damage.

## Methods and materials

*Bacteria*

*Prevotella intermedia* was isolated from a patient sample material, identified in the laboratory using standard diagnostic test systems (Remel Inc., Lenexa, KS, USA) and maintained by twice-weekly subculture in thioglycollate medium (Becton Dickinson and Co, Sparks, MD, USA). *Porphyromonas gingivalis* ATCC 33277 (Remel Inc.) was maintained by a twice-weekly subculture on CDC anaerobe blood agar (Becton Dickinson and Co.) and in thioglycollate medium (Becton Dickinson and Co.).

*Light and photosensitizer*

The source for light energy was a continuous working, high intensity 250-W Quartz Halogen light source, model I-250 supplied by Medithon (New York, NY, USA) that is used widely for ENT procedures in the office. The light was transmitted through a flexible light guide and filtered to maintain maximum energy output on the wavelength of 650 nm with broadband red filter (Edmund Optics Inc., Barrington, NJ, USA). The power density measured at 3 cm distance from the end of the light guide with filter was 50 mW $cm^{-2}$.

A 1% (w/v) methylene blue (MB) solution (Faulding Pharmaceutical Co., Paramus, NJ, USA) was used as a photosensitizer in all experiments. Subsequent dilutions of initial solution (to give 0.1, 0.075, 0.05, 0.025 and 0.01%) were prepared using sterile 10-ml bottles of normal saline and sterile syringes.

*Lethal photosensitization of* P. intermedia

Petri dishes containing CDC anaerobe blood agar (Becton Dickinson and Co.) were inoculated with 0.5 ml of broth containing $5 \times 10^5$ CFU $ml^{-1}$ and left closed at room temperature for about 10 min to let the broth penetrate the agar media. After that plates were exposed to 1 ml of MB solution at concentrations 0.1, 0.075, 0.05, 0.025 and 0.01% for at least 60 s and then exposed to high intensity red filtered light source at 1, 5, 10 and 20 min time intervals. Only plates with exposure to 0.01 and 0.025% MB were used in the experiment with 20 min light exposure. Four plates were used for each experiment. Four inoculated plates that were not exposed to MB or light source were used as controls. In order to examine the ability of light alone to cause bacterial killing, inoculated plates were exposed to the red-filtered light at the same time intervals without previous exposure to MB in the same groups of four plates. To study the ability of MB alone to produce bacterial killing, four inoculated plates were exposed to the MB solution at specified concentrations without subsequent exposure to the red-filtered high intensity light. Samples of the survivors from these procedures were taken from each plate with sterile 10 $\mu$l standard loop, dispensed in 1 ml of sterile NS and plated on to fresh media. Plates were incubated in anaerobic conditions in jars using anaerobic pack kits (BBL GasPak Plus; Becton Dickinson and Co.) at 37°C for 24 h and the resulting colony count was performed on each plate.

*Lethal photosensitization of* P. gingivalis

Serial dilutions of the *P. gingivalis* culture were prepared from the initial culture preserved on plates using sterile 10 $\mu$l standard loops (Becton Dickinson and Co.) and sterile NS. The Vitek colorimeter (Hach Company, Loveland, CO, USA) was used to achieve the final concentration of about $5 \times 10^3$ CFU $ml^{-1}$ (from an initial

**Table 1** Number of survivors (%) following exposure of *P. gingivalis* to different combinations of MB and light

| | *Light exposure (min)* | | | | |
|---|---|---|---|---|---|
| *MB (%)* | *0* | *1* | *5* | *10* | *20* |
| 0 (controls) | 75 ± 6 | 70 ± 6 | 75 ± 7 | 73 ± 8 | 71 ± 8 |
| 0.01 | 70 ± 11 | 73 ± 6 | 69 ± 7 | 55 ± 6 | 51 ± 4 |
| 0.025 | 75 ± 14 | 67 ± 4 | 47 ± 6 | 48 ± 8 | 49 ± 5 |
| 0.05 | 68 ± 12 | 53 ± 10 | 32 ± 7 | 32 ± 9 | Not attempted[a] |
| 0.075 | 51 ± 10 | 45 ± 5 | 26 ± 5 | 25 ± 5 | Not attempted[a] |
| 0.1 | 35 ± 9 | 39 ± 12 | 28 ± 9 | 24 ± 4 | Not attempted[a] |

[a]Exposure of cultures to combination of MB at different concentrations and light for 20 min (last column) was not attempted because significant killing rate was achieved with different combinations of MB and light with shorter time of exposure.

0.5 standard McFarland suspension – $10^8$ CFU $ml^{-1}$). Plates containing CDC anaerobic blood agar (Becton Dickinson and Co.) were inoculated with the resulting suspension using sterile 10 $\mu$l standard loops (Becton Dickinson and Co.). The plates were then exposed to the MB solution at different concentrations and thereafter to red-filtered high-intensity light source at different time intervals using the procedure described in the previous experiment. Plates were incubated at 37°C in the tightly closed jars supplied with anaerobic pack kits (BBL GasPak Plus; Becton Dickinson and Co.) for 24 h, and the resulting colony count was performed on each plate.

*Statistical analysis*

All statistical analyses were carried out using single-factor analysis of variance. Data are mean values ± s.d. of four values (Table 1).

## Results

No statistically significant reduction in bacterial counts was noted with the exposure of both cultures to the red-filtered high-intensity light source alone without the exposure to MB. No significant difference was noted between the exposure for 10 and 20 min in both species for all concentrations of MB. MB concentrations of 0.075 and 0.1% alone produced bactericidal effect without light exposure that proved to be statistically significant only for 0.1% concentration in *P. gingivalis*. With light exposure for 1 min both 0.075 and 0.1% concentrations of MB produced statistically significant bacterial colony count reduction for both *P. gingivalis* (Table 1) and *P. intermedius* (similar results; data not presented).

Statistically significant reduction in the bacterial colony count was achieved at concentrations of MB of 0.05% and higher and time of light exposure of 10 and 20 min for both species used in the experiment. Exposure of both cultures to red light produced statistically significant killing at the time intervals of 1, 5 and 10 min with MB concentration of 0.05% and at time intervals of 5 and 10 min with MB concentration of 0.025%. At MB concentration of 0.01% statistically significant killing was achieved only at 10 min light exposure.

## Discussion

The results of the study show that exposure of the named bacterial cultures to red-filtered high-intensity light for less than 10 min (with the accumulated energy level of 30 J $cm^{-2}$) in combination with exposure to MB solution at a concentration of 0.01% and higher produced significant bactericidal effect on both species examined. Significant reduction in bacterial counts can also be achieved with combination of light exposure for 5 min and MB concentration of 0.025% and light exposure for 1 min and MB concentration of 0.05% and higher. MB alone can produce bactericidal effect on *P. gingivalis* at concentration of 0.1%. Exposure to red light with wavelength of 650 nm alone does not produce any significant killing in *P. intermedia* and *P. gingivalis*.

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