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REVIEW ARTICLE

A POSSIBLE MECHANISM FOR THE BACTERICIDAL EFFECT OF VISIBLE LIGHT

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Visible light at high intensity was found to kill bacteria while low-power light in the visible and near infrared region enhances bacterial proliferation. The present review summarizes evidence demonstrating that the mechanism of visible light- bacteria interaction involves reactive oxygen species (ROS) generation. The ROS are photo induced by bacterial endogenous photosensitizers. Phototoxic effects were found to involve induction of high amounts of reactive oxygen species (ROS) by the bacteria while low amounts of ROS may promote their proliferation. Intense blue light, preferably at 415nm, is better than red light for bacteria killing.

Key Words: Visible Light, Bacteria Killing, Reactive Oxygen Species (ROS)

Introduction

The traditional approach for destroying bacteria is mainly antibiotic drugs which are not very efficient because of the development of resistant species. In addition the limited penetration of drugs into bacterial biofilm results in reduced susceptibility to this kind of treatment. Obviously there is a growing need for innovative approaches leading to bacteria eradication. One area of interest involves the use of light-based treatment technologies. UV irradiation is well-known to photo-destroy bacteria and other microbes but even minimal overexposure to UV is dangerous to the healthy tissue.

Recently there are several reports on the bactericidal effect of visible light, most of them claiming the blue part (400-500nm) to be responsible for killing various pathogens. For example Feursteinet et al., 1) showed that broadband blue light sources at 400-500nm exert a phototoxic effect on *P. gingivalis* and *F. nucleatum*, and Henry et al., 2) demonstrated that low fluencies of argon laser irradiation (488-514 nm) exert a phototoxic effect on *Porphyromonas* and *Prevotella* spp., which are both Gram negative anaerobic bacteria that produce porphyrins. Oral black-pigmented bacteria (BPB) in pure cultures and in dental plaque samples were killed by 4.2 J/cm^2 blue light, whereas *P. melaninogenica* required 21 J/cm^2. 3) *Propionibacterium acnes* was also inactivated by blue light without an exogenous photosensitizer. 4,5) Investigations using a high-intensity xenon lamp, 6) have demonstrated the sensitivity of *S. aureus* (a non pigmented bacterium) to visible light, and also identified the bactericidal wavelengths inducing maximum visible-light inactivation to within a 10 nm bandwidth. Their results have highlighted that inactivation is evident using 400 – 420-nm-wavelength blue light, with the most effective bactericidal activity at 405 ±5 nm. Maclean *et al.*, showed that an 405 nm LED array has a phototoxic effect on a variety of bacteria including Gram-positive bacteria: *S.*

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aureus – MRSA, S. epidermidis, S. pyogenes, C. perfringens; Gram-negative bacteria: *A. baumannii, P. aeruginosa, E. coli, P. vulgaris* and K. *pneumoniae.* 7)

Wavelengths of longer than 430nm were found to induce no effect on the viability of *S. aureus* cells. These results are in contrast to those of Chukuka 8) and Guffey 9) who found a significant killing effect of S. aureus at 470nm. Also enteric bacterial species and *Helicobacter pylori* were found to be sensitive to visible light illumination. 10-12)

There are some authors claiming bacteria killing with red and near IR light. For example Nussbaum et al. 13) reported a bactericidal effect at 630 nm for *Pseudomonas aeruginosa* and *E. coli.* We have found that even high power 780nm diode laser (100mW/cm^2) did not kill *S. aureus.* 14) Combination of blue and red light was found by Guffey JS *et al.* to be effective against *S. aureus* and *P. aeruginosa.* 15)

Opposite to visible light induced inactivation of bacteria, an elevation in bacterial viability following illumination using low power light was observed (Dadras *et al.*, 16) Karu *et al.*, 17) Polo *et al.*, 18) Nussbaum *et al.*, 13,19) Lipovsky *et al.* 14)). This is not surprising since a stimulatory effect of low energy visible light irradiation on various cells proliferation have been largely demonstrated in vitro in a variety of cell lines. 20,21)

There are few works attempting to explore the mechanism of the bactericidal effect exerted by visible light. Chukuka *et al.* 22) believe that blue light exerts similar effects on DNA as ultraviolet 20) light, being absorbed in the double bond within the pyrimidine bases of DNA such as thymidine and cytosine. 23)

In the present review we summarize evidence suggesting that the bactericidal effects of visible light could be attributed to high amounts of reactive oxygen species (ROS) generated by endogenous photosensitizers in the bacteria.

Visible light induced ROS in bacteria

Reactive oxygen species (ROS) include oxygen radicals, singlet oxygen and peroxides. They are generally very small molecules and are highly reactive due to the presence of unpaired valence shell electrons.

It is known that high amounts of ROS are lethal to the cell, a phenomenon exploited in photodynamic therapy (PDT), which is typically employed for cancer therapy and antibacterial treatment. PDT employs **exogenous photosensitizers**, such as hematoporphyrin derivatives, which are introduced to the cells and then irradiated with an appropriate wavelength of visible or near infra-red (NIR) light. The activated photosenstizer molecules pass on their energy to surrounding molecular oxygen, resulting in the formation of ROS. In the past we showed, that light in the **visible range** is capable of generating **ROS in living cells** following its absorption by **endogenous cellular photosensitizers** such as cytochromes, porphyrins, flavins and NADH. 24) The endogenous cellular photosensitizers have broad absorption bands over the entire visible range with a maximum in the blue region. 25) As bacteria also possess **endogenous photosensitizers** we have suggested 26) that high intensity visible light could generate high amount of ROS thus leading to bacteria killing. Bacteria which possess high amounts of **endogenous photosensitizers**, like for example *Propionibacterium acnes*, can easily be destroyed with visible light. Moreover, two different strains of the same bacteria which were found to differ in their endogenous porphyrin content and their antioxidant activity responded differently to visible light. 14)

The involvement of oxygen in the phototoxic effect of visible light on bacteria 27,28) and the inhibition of the phototoxic effect following addition of various scavengers to bacterial suspensions before exposure to light, 29,30) also support the hypothesis that the bactericidal effect of visible light involves photo-oxidative reactions.

In the following paragraph direct evidence showing ROS generation in illuminated bacteria is shown.

1. Direct Detection of ROS in illuminated bacteria

A very useful technique for detecting ROS in illuminated bacteria is the EPR spin trapping measurement. Since ROS have a very short half-life time (ns-ms), making them very difficult to detect directly, a diamagnetic compound, a spin trap which binds the ROS, is added to the bacteria. The resulting long-lived free radical called a spin adduct, is then detected by the EPR technique. As each radical has a different hyperfine structure, this technique is a powerful tool to identify specific radicals. DMPO is a common spin probe that detects $^\bullet$OH to give the spin adduct DMPO-OH (Eqn 1) that gives a quartet EPR signal.

(1) DMPO+$^\bullet$OH → $^\bullet$DMPO-OH

DMPO can also trap $O_2{\cdot}^-$ to produce the spin adduct DMPO-OOH. Nevertheless, since the latter is unstable, it decomposes to DMPO-OH adduct 31,32)

In **Figs.1 and 2** the EPR spectra of white light illuminated *E. coli 1313* and two strains of *S. aureus* (*101* and *500*) are shown. The four peaks characteristic of

DMPO-OH adduct, can be assigned to formation of hydroxyl and or superoxide radicals.

Recently we have found that a very sensitive method for measuring free radical production is by the observation of the decay of the triplet EPR signal of 2, 2, 6, 6-tetramethyl piperidine-*N*-oxyl (TEMPO). A detailed description of the advantages of the nitroxide TEMPO over the more popular EPR spin trap 5,5 DMPO was given in our previous publication. [25] In **Fig. 3** the reduction of the triplet signal of TEMPO

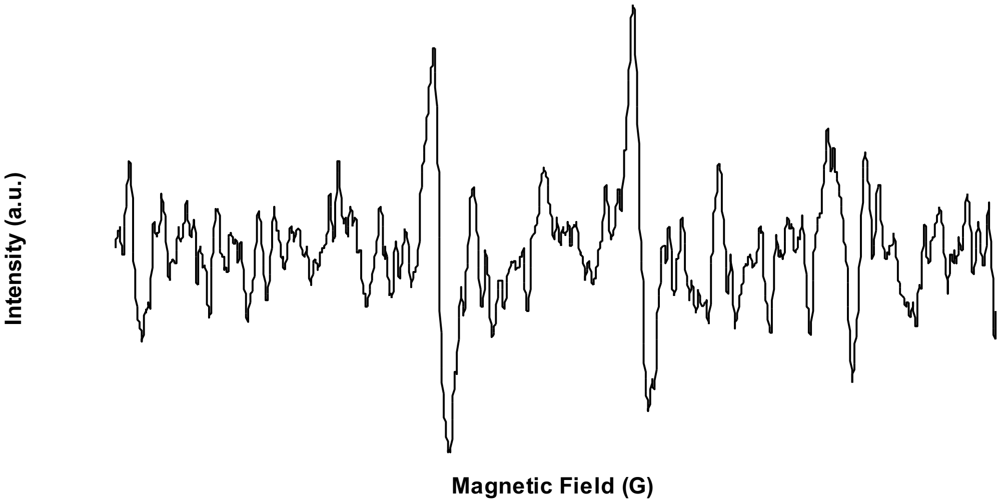

Fig.1: ROS formation in irradiated *E. coli 1313*. [33]

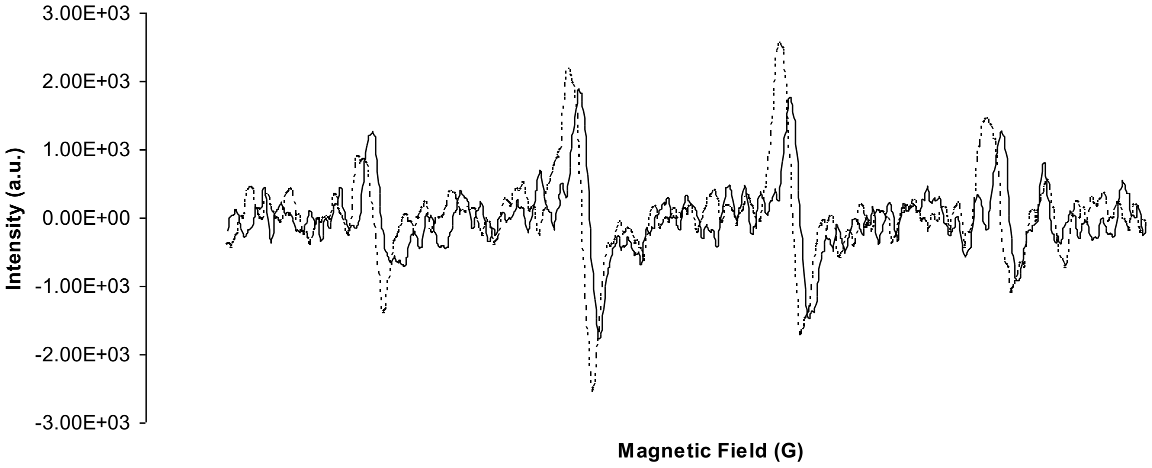

Fig.2: ROS formation in irradiated *S. aureus 101* (dotted line), *S. aureus 500* (solid line). [14]

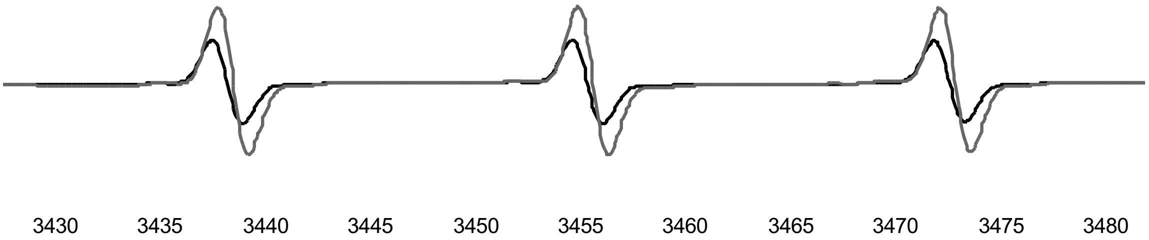

Fig.3: ROS formation following blue light (400-500nm) illumination of TEMPO in the presence of *E. coli.* [34] Gray line-before irradiation, black line- after irradiation

(black line) following blue light (400-500nm) illumination of TEMPO in the presence of *E. coli* is demonstrated. The reduced intensity of the TEMPO signal indicates the production of ROS in illuminated *E. coli*.

It is important to note that the amount of ROS produced in illuminated bacteria was in correlation with the phototoxic effect. [14,34] ROS production by two different *S. aureus* bacterial strains as measured by the EPR technique was in correlation with each strain's different sensitivity to visible light. *S. aureus 101*, which was destroyed by light, produced more ROS than *S. aureus 500*, which is more resistant to light and produced smaller amounts of ROS. [14]

In light of the previous literature and the direct EPR measurements, it can be concluded that the phototoxic effect of visible light is a consequence of light induced ROS in the bacteria.

2. The priority of blue light in inducing ROS formation in bacteria

To determine the optimal wavelength for ROS generation in bacteria, several bacteria were illuminated with various visible wavelengths and the EPR spectra were measured. ROS production following blue (400-500nm) light illumination was found to be much higher than that of red (500-800nm) (see **Fig.4**) which means that blue light is much more effective for killing bacteria.

Within the blue range, light of 415nm induced more ROS than 455nm, which correlates with results obtained for the reduction in colony count of *S. aureus and E. coli* following illumination using equal intensities of these two wavelengths. [34]

Summary:

In the present review we have summarized evidence demonstrating that the mechanism of visible light toxic effects on pathogens involves ROS generation. The ROS are photo induced by endogenous photosensitizers. Bacteria rich of photosensitizers or possessing low amounts of antioxidants will be more sensitive to light. However, it should be noted that low intensity visible light can be dangerous since it may promote proliferation of bacteria by generating low amounts of ROS that has been found to induce cell growth.

Intense blue light, preferably at 415nm, is better than red light for bacteria killing.

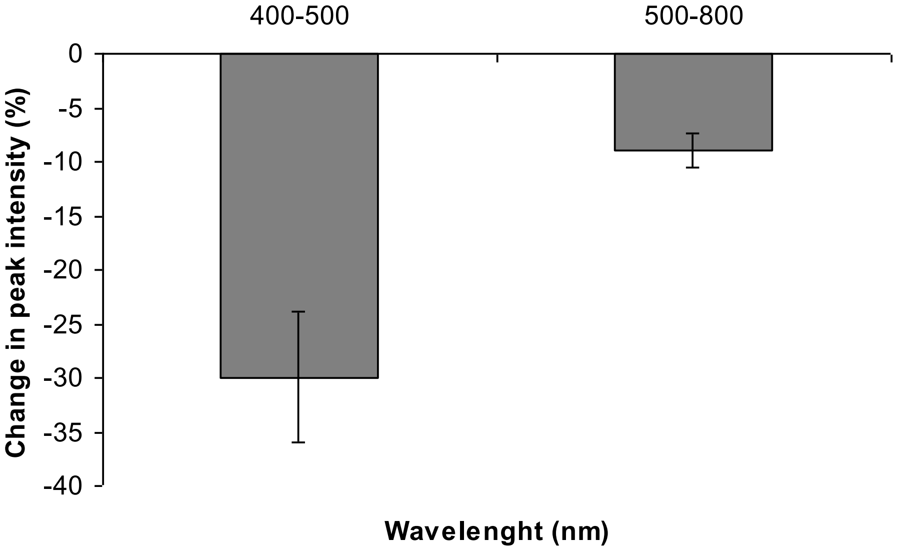

Fig.4: Change in TEMPO signal intensity (which is proportional to ROS formation) following illumination with blue (400-500nm) or red (500-800nm) visible light. [34]

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