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The Inhibition of *Streptococcus mutans* by He- Ne Laser via TBO Photosensitizer

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Abstract: This work aims to investigate the inhibition of vitality of *Streptococcus mutans*, which is the causative agent of caries. A 632.8 nm He-Ne laser with the output power of 4.5mW was used in combination with toluidine blue O (TBO) at the concentration of 50µg/ml as a photosensitizer. *Streptococcus mutans* was isolated from 35 patients if carious teeth. Three isolates were chosen and exposed to different energy densities of He – Ne laser light 3.8, 11.7, 34.5 and 104.1 J/cm². After irradiation, substantial reduction was observed in the number of colony forming units (CFU)/ ml. The reduction in the number of CFU was increasing as the dose increased.

Introduction

Since the invention of laser forty years ago, their field has been developed rapidly and applications of laser have been expanded from pure physics to biology, technology, chemistry, medicine and allied field (Chopra and Shawla, 1990).

Dental caries is a chronic invasive disease of dental tissue. The bacteria that are responsible include *Streptococcus mutans*, *Streptococcus sobrinus*, *Lactobacillus casi and Actinomyces viscosus.*(Burns *et al.*,1996). Current methods of treatment are relatively crude and involve mechanical removal of sound tooth tissue, to gain access to the lesion, as well as the removal of softened and infected dentine. It would be advantageous, if the bacteria could be killed in situs, reducing the amount of tissue needing to be removed. This could be achieved by the use of the lethal photosensitizer agent, e.g. various dyes, thus rendering them susceptible to killing by light from low power lasers. (Burns *et al.*, 1996).

Although little attention has been given to bactericidal effect of laser radiation particularly using low-power lasers, it has demonstrated that He-Ne laser light has inhibitory action on cariogenic bacteria (Okamoto *et al.*, 1992).

The main purpose of this study is to investigate the inhibition action of He – Ne laser with photosensitizer (TBO) on *Streptococcus mutans*, which is the main causative agent of caries teeth.

Materials and Methods

Sample collection

Samples collection was done by using small swabs. Thirty –five samples were collected from patients have caries teeth from clinics.

Isolation of Streptococcus mutans (S. mutans)

After collecting the samples from caries lesions of patients teeth, they were grown on one of the selective media mitis salivarius agar (MSA) and mitis salivarius bacitracin agar medium (MSBA) and incubated anaerobically by using anaerobic jar for 48hrs at 37ºC.(Hardie, *et al*.,1986).

Identification of S. mutans

The identification of bacteria includes microscopical examination and biochemical tests as follows:

Microscopical examination

The cell morphology examination includes cell arrangement, formation of chain and Gram stain method (Collee *et al.*, 1996).

Biochemical examination

According to the Sneath *et al.*(1986) and Holt *et al.*(1994), blood hemolysis, catalase production and carbohydrate fermentation tests were done to identify *S. mutans.*

Blood hemolysis test

Include inoculation of bacterial samples on blood agar (Sneath *et al.*1986).

Catalase production test

Catalase activity of *S. mutans* was tested by picking a small amount of tested colonies from agar by a wood stick and mixed it with few drops of H_2O_2 (3%) on glass slide. The absence of gas bubbles formation indicates the absence of catalase activity (Wittenbury, 1964).

Carbohydrate fermentation test

One colony of *S. mutans* culture activated in Brain Heart Infusion Broth (BHIB) for 18hr were inoculated in tubes containing sterile fermentation medium supplemented with the tested sugars (sorbitol and mannitol). A blank tube contained the fermentation medium without the bacterial inoculum was also included. All tubes were incubated for 5 days at 37ºC, the results were recorded daily when the phenol red indicator color changed from red to yellow (Collee *et al.*, 1996).

He: Ne laser and photosensitizer

A He: Ne gas laser was used in this investigation. Its power was 4.5 mW, emitting the light with a beam diameter of 3 mm at a wavelength of 632.8 nm in a continuous mode (CW). The exposure time varied to get different energy densities. This laser was used in a conjunction with the photosensitizer toluidine blue O, which has maximum absorptions. The energy density was obtained from

Energy density (J) =Exposure time (min.). Laser output power (W) / Area (cm^2).

He: Ne laser Irradiation with TBO

Streptococcus mutans was maintained by subculturing on blood agar (human blood) every seven days. One colony or more than one grown on BHIB for 18 hrs at 37ºC in anaerobic jar. Then the culture was centrifuged at 3500 r.p.m for 10 min. The supernatant was removed and the bacterial pellet re-suspended using phosphate buffer saline (pH=7) and homogenate by wring vortex mixer. The suspension was diluted to obtain a concentration of 10^5 colony forming unit (CFU) /ml concentration then 0.5 ml of this suspension was introduced in each Eppendroff tube. A 0.5 ml of photosensitizer (TBO) was added to these tubes then exposed to laser light at different exposure times, (with exception of one used as a control), suspensions in Eppendroff tubes were grown on MSA and incubated anaerobically for 24 hrs. The colony forming units (CFU) were enumerated, (this represents the survival colonies).

Results and Discussion

The results of bacterial identification were get using the microscopical examination and biochemical tests, where in the microscopical examination, the colonies of *Streptococcus mutans* appeared rough heaped colonies, about 0.5 mm in diameter, often with beads droplets of liquid containing soluble extracellular polysaccharides on or around the colonies and tending to adhere to the surface of the agar, other formed smooth, mucoid colonies while the cells of *Streptococcus mutans* on gram stain slide appeared spherical or avoid, positive grame staining cocci, arranged in pairs, short medium or long chains, without capsule, non- spore forming, non-motile cocci. The results of bacterial irradiation by He: Ne laser in the existence of the photosensitizer at different energy densities (3.8, 11.7, 34.5 and 104.1) J/cm^2, are shown in figures (1, 2 and 3).

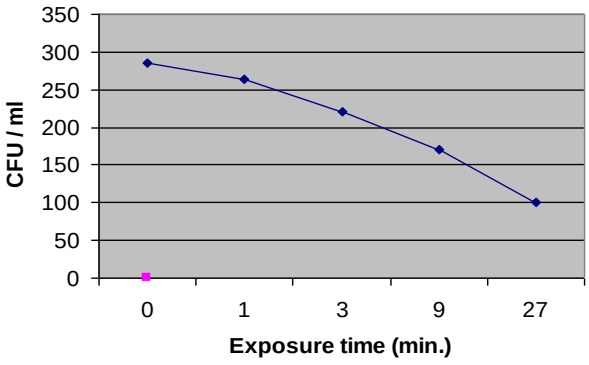

Fig. (1) The effect of He – Ne laser in the presence of TBO on *S.mutans* isolate no. 1

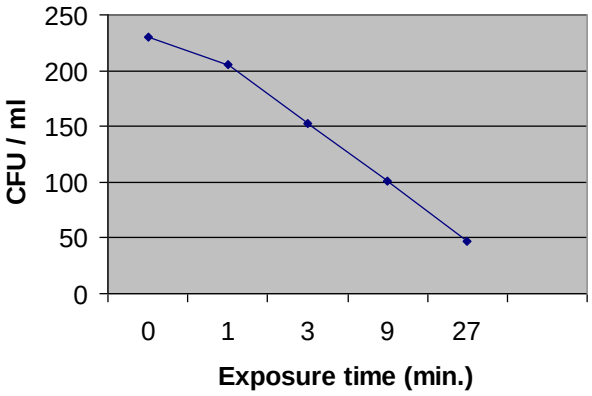

Fig. (2) The effect of He – Ne laser in the presence of TBO on *S.mutans* isolate no. 2

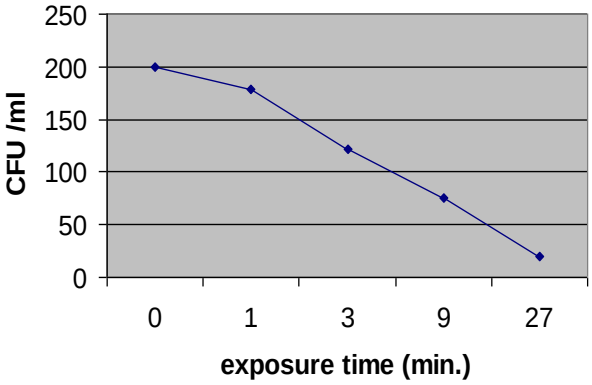

Fig. (3) The effect of He – Ne laser in the presence of TBO on *S.mutans* isolate no. 3

The colony forming unit as a function of exposure time, shown that there is a significant decrease in CFU as the energy density increase (the percentage of killing *S. mutans* increased as the energy density increased as shown in figures 4, 5, and 6).

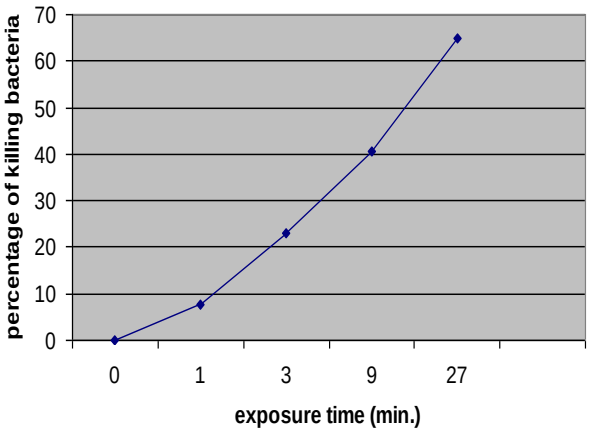

Fig. (4) The percentages of killing bacteria isolate no. 1

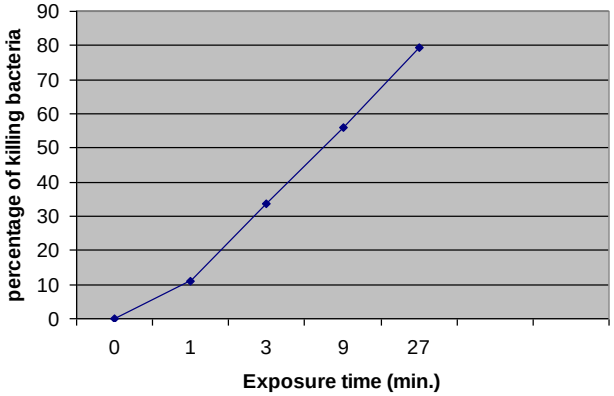

Fig. (5) The percentages of killing bacteria isolate no. 2

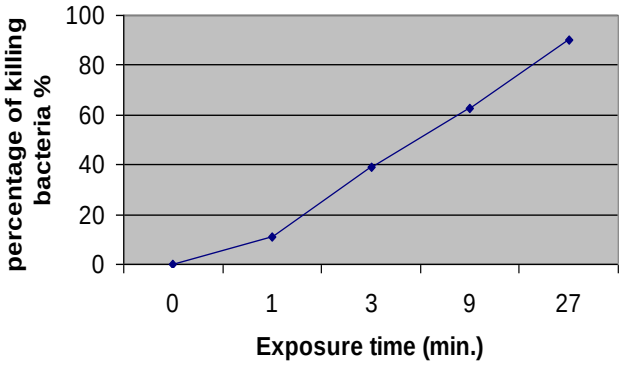

Fig. (6) The percentages of killing bacteria isolate no. 3

This indicates that the bacterial effect depends on the energy density. It could be interpreted as the cariogenic bacteria and other plaque forming organisms can be killed by low power laser light in the presence of a suitable photosensitizer (Wilson *et al.*, 1992; Wilson and Dobson, 1993, Hassan, 2003; Zanin *et al*., 2005). Hence, *S. mutans* and *S. sobrinus*, could be killed by light from He: Ne laser light in the presence of TBO (Burns *et al.*, 1993).when a photon is absorbed by a certain molecules (known as photosensitizer), it is promoted to a high – energy state (known as the triplet state) which then transfer its energy to an oxygen molecule resulting in the generation of singlet oxygen (which is cytotoxic) (Walsh, 2003). This mechanism can be used to kill bacteria in a process termed lethal photosensitizer or photodynamic therapy (PDT), which is non-cumulative, local treatment and considered as an appropriate antimicrobial strategy for the treatment of local infections. Because of a widespread development of antibiotic resistant

strain of medically – important bacteria (Doughetty *et al.*, 1998), the ability of TBO to sensitize *S.mutans* to killing by low power laser light as demonstrated in this study may have clinical applications, if effective in vivo, in that it could be used to kill these organisms in a carious lesion prior to its repair.

Caries is a disease well suited to PDT as it is localized infection and so there would be need for systematic administration of the photosensitizing agent, thus reducing the possibility of toxic side effects.

Table (1) Biochemical test of *S. mutans*

Biochemical test / bacteria	Fermentation		Blood hemolysis	Catalase test
	Mannitol	Sorbitol		
S. mutans Iso. No.1	+	+	Non	_
S. mutans Iso. No.2	+	+	Non	_
S. mutans Iso. No.3	+	+	Non	_

The sensitizers could be applied to the lesion and localized to the lesion only by means of syringe and the laser light could then be delivered via an optical fiber. This approach to kill bacteria offers several advantages over the use of conventional antimicrobials. Firstly, development of resistance to the photochemical generated free radical responsible for bacterial killing (MacRobert *et al.,* 1989; Malik *et al.,* 1990) would be unlikely. Second unlike the suspension with antiseptic and antibiotic, there would be no need to maintain high concentration of the photosensitizer in the lesion for long period of time. The necessary residence time of the photosensitizer would be no more than few minutes. The main advantage of killing cariogenic bacteria in the situ is that it would be obviate the need for mechanical removal of infected dentine, thus enabling restoration of the lesion with minimal removal of the repairing tooth.

Conclusion

The results of this *in vitro* investigation have demonstrated that *S. mutans* can be killed by red light from a low – power laser after having been sensitized with TBO.

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القضاء على البكتريا Streptococcus mutans باستخدام ليزر الهليوم: نيون والمتحسس الضوئي TBO

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الخلاصة

تهدف الدراسة الى البحث في امكانية القضاء على البكتيريا نوع Streptococcus mutans المسببه للتسوس . لقد تمت محاولة الربط بين مؤثرين هما ليزر الهليوم نيون بقدرة 4,5 ملي واط مع الصبغة نوع تلودين–بلو (TB6) بتركيز 50 مايكروغرام / مليلتر كمتحسس ضوئي. تم عزل البكتريا من 35 مريضا مصاب بتسوس الأسنان وقد أختيرت ثلاثة عينات للدراسة . عرضت العينات الى جرعات مختلفة من الليزر وهي 3.8, 11.7, 35.5, 104.1 جول/سم^2. أظهرت النتائج نقصا واضحا في عدد الوحدات المكونة للمستعمرات وهذا النقصان يزداد مع زيادة جرعة الليزر .