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Investigation of Bactericidal Effect of Low Level Laser of Galium-Aluminium-Arsenide on Cariogenic Species of *Streptococci* and *Lactobacillus*

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The aim of present *in vitro* study was to investigate the antibacterial effect of low level laser of Galium Aluminium Arsenide (GaAlAs) on cariogenic bacteria. Suspensions of *Streptococci mutans*, *Streptococci sanguis*, *Streptococci subrinus*, *Streptococci salivarius* and *Lactobacillus* sp. were exposed to a GaAlAs (890 nm) in the presence of photosensitizer toluidine blue O in different time points of 2, 5, 12, 16 and 20 min. Viable microorganisms were counted on muller hinton agar plates after overnight incubation at 37°C and reported as colony forming unit. For all tested bacterial strains, at time points of 5, 12, 16 and 20 min, there was a significant decrease in the viable counts, reaching to a maximum value of 100% after 20 min, when treated with combination of laser + TBO (toluidine blue O). The combination of laser and TBO had the least effect on *Lactobacillus* sp. in time points of 5, 12 and 16 min, whereas it was more effective against *S. mutans* and *S. sanguis* at the same time points. Based on the presented results, we may conclude that the low level laser of GaAlAs was effective in significantly reducing of the viability of oral cariogenic bacteria *in vitro*.

Key words: Low level laser, cariogenic species, *Streptococci*, *Lactobacillus*

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INTRODUCTION

The oral cavity of man is colonized by very large numbers of a highly diverse community of bacteria as many as 1000 different non-harmful microbial species (Wilson, 2004). There is also a limited number of acid-producing bacteria in the oral cavity called cariogenic bacteria, that have been identified as the aetiological agents of a variety of diseases both within and outside of the oral cavity (Kononen, 2000). These bacteria utilize sucrose to synthesize extracellular polysaccharides called glucans, which allow them to adhere to the tooth's surface and form the dental plaque or biofilm (Marsh, 2003). Cariogenic bacteria are usually found in small quantities in healthy plaque. However, with biological and environmental perturbations, these bacteria may become dominant in the oral flora and produce large amounts of acid in the dental plaque due to fermentation of carbohydrates such as sucrose, fructose and glucose (Hamada, 2002). The resulting high levels of acidity from lactic acid in the mouth affect teeth because a tooth's special mineral content causes it to be sensitive to low pH. When the pH at the surface of the tooth drops below 5.5, demineralization proceeds faster and results in the ensuing decay. In addition to dental caries, the accumulation of these bacterial biofilms on tooth surfaces results in inflammatory periodontal disease (Wilson, 2001).

Periodontal diseases are among the most prevalent infectious diseases of man, with 75% of 35-44 years old being affected-the proportion increases with age and 95% of those over 65 years of age are affected (Burk and Eklund, 1999).

The organisms in supragingival plaque considered to be the major aetiological agents of caries are *Streptococcus mutans*, *Streptococcus sobrinus*, various *Lactobacilli* and *Actinomyces viscosus* (Wilson, 2004). *Streptococcus mutans* has been implicated as the primary caries-causative agent because it presents in relatively high numbers in plaque prior to the appearance of carious lesions and its ability to rapid degradation of carbohydrates and induce a tolerance to low level pH environments (Zanin *et al*., 2005).

A comprehensive diagnosis of dental caries should include the detection of cariogenic bacteria, the survey of plaque acidogenicity and the recognition of tooth demineralization sites (Steinberg, 2007). Current treatment regimes for plaque-related diseases involve the mechanical removal of the causative organisms. In the case of caries, this consists of drilling away the infected enamel and dentine while periodontal diseases are treated by removal of plaque accumulating at the gingival margin and subgingivally (Jorgensen and Slots, 2000; Caufield *et al*., 2001). As microbial plaques have been proven to be the primary aetiological agent of inflammatory periodontal disease, the major purpose of periodontal therapy has been to eliminate all bacterial deposits on the tooth surface by use of systemic antibiotics. However, overuse of antibiotics has been a major problem in the production of drug-resistant organisms (Haper *et al*., 1999). Therefore, the application of an alternative method to eradicate bacteria from periodontal pockets is desirable. One such approach is photodynamic therapy.

In 1904, Jodlbauer and von Tappeiner, first successfully demonstrated the photodynamic inactivation of bacteria by an exogenously applied photosensitiser (Chan and Lai, 2003). Light from high-power lasers is known to be bactericidal and investigations have shown that it is effective against organisms implicated in caries and inflammatory periodontal diseases. However the adverse effects of such light on dental hard tissues argue against its use solely as an antibacterial agent (Wilson, 2004). Recently, a series of studies have shown that it is possible to kill bacteria with a light source from a low power laser after the microorganisms have been sensitized with a low concentration of dye, such as methylene blue (MB) or toluidine blue O (TBO) with no adverse effect (Chan and Lai, 2003). The purpose of this *in vitro* investigation was to evaluate the antimicrobial effect of low level Galium Aluminium Arsenide (GaAlAs) laser on some of the bacteria involved in dental caries.

MATERIALS AND METHODS

The study was conducted from June to December 2006. The bacterial strains used in this study were: *Streptococci mutans* (ATCC 25175), *Streptococci sanguis* (ATCC 10556), *Streptococci subrinus* (ATCC 6715), *Streptococci salivarius* (ATCC 13419), *Lactobacillus* sp. (ATCC 4356). All standard strains were purchased from Mast Company, Germany and IROST, Tehran.

The bacteria were subcultured on blood agar plates (Merck, Germany) and were incubated at 37°C in presence of 10% CO_2 for 24 h. An overnight culture were prepared in thioglycollate broth (Merck, Germany) by transferring a few colonies grown on blood agar plates. The bacterial suspensions were then diluted in broth to an optical density of McFarland No. 1 (approximate numbers 3×10^8 bacteria mL^{-1} (Forbes *et al*., 2007). A 2 μL sample of adjusted suspension was mixed with 1 μL of toluidine blue O (TBO; Sigma Ltd., UK) as photosensitiser to a final concentration of 0.01% w/v and was put into the wells of a flat bottom 96-well microtitration plate in triplicate. The microplate was put in dark room on a microplate shaker for 5 min before irradiation.

Irradiation was performed using a gallium aluminium arsenide (GaAlAs) diode laser (Omega Universal Technologies, London) was used in this study with a power output of 10 mW at a wavelength of 890 nm in a pulsed mode with a frequency of 20 kHz.

The delivery of laser energy was 2, 5, 12, 16 and 30 min. The distance between the laser fiber and sample was 5 mm approximately (Chan and Lai, 2003). Negative controls untreated by either lasers or photosensitiser (TBO) were included in the study as well (control 1). Besides, for investigation the effect of combination of dye and laser, additional controls were prepared by TBO plus each sample to a final concentration of 0.01 w/v untreated by laser (control 2) and samples exposure to laser without adding TBO (control 3).

After irradiation, 1 μL of each suspension was mixed with 9 μL of thioglycollate broth and incubated for 2 h at 37°C. The triplicate concentrations of each bacterial suspension (0.1, 0.2, 0.3 mL) were then subcultured on muller hinton agar (Merck, Germany) and incubated at 37°C in presence of 10% CO_2 overnight and viable microorganisms grown on the plates were counted and converted into colony forming units (cfu) in the next day.

The Statistical Package for Social Sciences (SPSS Inc., Chicago, IL, USA) was used for data analysis.

RESULTS

The colony counts were calculated by accounting dilution factor of 1:1000 for five bacterial strains. No significant effect on viability of tested bacteria was seen with 2 min radiation with energy density of 3.6 J cm^{-2}. The cfu of bacteria in this time point lasing, was equal to what was seen in negative control groups.

For all tested bacterial strains, at time points 5, 12, 16 and 20 min, there was a significant decrease in the viable counts when treated with combination of laser + TBO.

As seen in the Table 1, during a 5 min exposure to laser (energy density = 9.0 J cm^{-2}) the average bacterial death rate was 12%. By increasing the exposure time to 12 and 16 min the average bacterial death rates went up to 33.6 and 48.6%, respectively (energy densities = 21.6 and 28.8 J cm^{-2}).

The reduction of viable count of all tested bacteria reached a maximum value (100%) after 20 min exposure to laser (energy density = 36 J cm^{-2}) + TBO. The combination of diode laser and TBO had the least effect on *Lactobacillus* sp. in time points of 5, 12 and 16 min, whereas it was more effective against *S. mutans* and *S. sanguis* at the same time points, but it was not much significant.

The effect of TBO alone on tested bacteria was minor and in average only 2-5% reduction was seen in viability of bacteria. Besides, in the absence of TBO, the GaAlAs laser did not lead to significant decrease in the viable count. The control group of bacteria which were exposed to 20 min laser alone, showed reduction in viability of bacteria comparable to 5 min laser +TBO effect.

DISCUSSION

Due to the emergence of antibiotic resistance, photodynamic therapy (PDT) has become a viable alternative antibacterial therapy for biofilm-related diseases such as dental caries. The advantages of photodynamic therapy over conventional antimicrobial agents are first, rapid killing of target organism in a few min depending mainly on the light energy dose delivered, in contrast with hours or even days necessary in the case of conventional antimicrobial agents and second, antimicrobial effects can be confined to the site of the lesion by careful topical application of photosensitizer and the area of irradiation can be restricted further by using an optical fibre (Jorgensen and Slots, 2000). Based on these advantages, several studies were undertaken using PDT and have shown that oral bacteria are susceptible to PDT (Haas *et al*., 1997; Korytnicki *et al*., 2006; Müller *et al*., 2007).

The results of this study showed that exposure of bacterial cultures to laser light in the presence of TBO as a photosensitiser results in a light energy dose-dependent decrease in viability. We have chosen TBO as photosensitizer based on other investigations which revealed TBO as the most effective photosensitizer (Wilson, 2001).

The most-effective combination was that of TBO with a 890-diode laser at 100 mW with light energy density of 36 J cm^{-2}, when used for 20 min. This produced a 100% kill rate in the various species that were tested. To exclude the possibility that the absorption of laser energy in TBO may raise the temperature to kill bacteria, a pretest was

Table 1: Susceptibility of oral cariogenic bacteria to GaAlAr laser plus photosensitizer (TBO)

	Viable count colony forming unit (viability reduction %)				
Bacterial strains	2 min	5 min	12 min	16 min	20 min
S. mutans	NC	8.54×10^6 (15)	6.54×10^6 (35)	4.53×10^6 (55)	0(100)
S. sobrinus	NC	8.54×10^6 (15)	6.03×10^6 (40)	5.09×10^6 (50)	0(100)
S. salivarius	NC	8.72×10^6 (13)	6.54×10^6 (35)	5.37×10^6 (47)	0(100)
S. sanguis	NC	9.03×10^6 (10)	6.25×10^6 (38)	5.23×10^6 (48)	0(100)
Lactobacillus sp.	NC	9.03×10^6 (10)	7.04×10^6 (30)	5.75×10^6 (43)	0(100)

NC: No change in viability

done to evaluate the thermal effects. The results showed that the minor temperature increased in all lasing group with or without TBO. So we concluded that as other studies were suggested for methylene blue as sensitizer, TBO did not convert laser energy into heat that may kill microorganisms under the test conditions (Chan and Lai, 2003). In general, the ability of the laser light to kill the cariogenic bacteria was dependent on the duration of irradiation. Among the tested streptococci, *S. mutans* and *S. sobrinus* were the most sensitive bacteria to irradiation. This is a promising *in vitro* findings, since these are among the predominant bacteria are normally found in dental plaque.

Although the results of this *in vitro* study have shown that tested cariogenic bacteria can readily be killed by the appropriate laser-dye treatment combination and was in agreement with most of the similar research studies which have been conducted *in vitro*, further *in vivo* evaluation is necessary to evaluate recolonization of the microorganisms once laser treatment was finished.

Based on the results presented in this study we may conclude that the combination of laser and TBO was effective in significantly reducing of the viability of oral bacteria as other investigations concluded (Haas *et al*., 1997; Korytnicki *et al*., 2006; Müller *et al*., 2007). However there are studies in controversy of ours that reported the antibacterial effect of laser in the absence of a photosensitizer (Nussbaum, 2002).

In conclusion, based on the presented results, we may conclude that the low level laser of GaAlAs was effective in significantly reducing of the viability of oral cariogenic bacteria *in vitro*.

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