Effects of Soft Ga-As Laser on Streptococcus Mutans and on Skin and Mucosa Temperature Changes

Učinak Ga-As lasera na Streptococcus mutans i temperaturne promjene na koži i sluznici

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Summary

Effects of the Ga-As soft laser on clinically oral-isolated Streptococcus mutans and on temperature changes of the finger pad and ear lobe skin and tongue mucosa were studied. Bacterial suspensions of S. mutans were placed on solid blood agar plates. The center of the plates was subjected to 3-min laser treatment every 24 h during a six-day period. Then the bacteria from the target area were maintained on a new plate and examined for survived bacteria. The use of the laser on the agar plate surface produced no bacterial or bacteriostatic effect. Additionally, temperature changes of the ear lobe skin and finger pad skin and tongue mucosa during and after laser treatment were not significantly different from the control values.

Key words: *antibacterial effect, temperature changes, l aser*

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Introduction

The use of soft lasers to improve conventional oral therapy has dramatically increased in recent years. According to their output power, lasers are divided into two major categories, hard and soft lasers. Hard lasers are energy devices that cut, vaporize or carbonize the target tissue. So-called soft lasers are low-energy devices, and therapy involving this type of laser has been recommended for diverse conditions, like periodontal and oral disease (1), anticariogenic measure (2) and postoperative pain (3). However, the role of soft lasers in wound healing and the treatment of pain has not yet been established. Wakabayashi et al. (4) have reported that low power laser irradiation has a suppressive effect on injured tissue by blocking the depolarization of C-fiber afferents.

Re and Viterbo (5) have reported that low energy laser has antimicrobial effect, however, they refuse to speculate on the mechanism of antimicrobial action of soft laser. It has been known that bacteria can be killed by exposure to UV light, however, Malik et al. (6) have recently shown that bacteria can be killed by visible light after they have been treated with a photosensitizer. The sensitizing agent could be applied topically and delivery of the laser to the target area would present no difficulties (7). Burns et al. (8, 9) have reported that cariogenic bacteria can be killed by He-Ne and GaAlAs soft lasers following sensitization with toluidine blue and aluminum disulphonated phthalocyanine, respectively.

Previous studies have reported on a very small effect of heat produced by low power laser (10). In this study, a temperature rise of about 0.5 °C during irradiation with low power laser was recorded inside the pulp chamber. Paschoud and Holz (11) have also reported that the intrapulpal thermic control, accomplished in vivo, revealed a ±0.5 °C variation after 2-min irradiation with He-Ne laser. In recent literature, there is no data on temperature changes at the site of soft-laser impact spot during and after soft-laser irradiation.

The present study was, therefore, designed to investigate the antimicrobial effect of gallium-arsenide (Ga-As) laser on oral *Streptococcus mutans* and, in addition, the effect on temperature changes on the impact spot, recorded on the surface of ear lobe, finger pad and tongue mucosa during and after laser treatment.

Material and Methods

Laser devices

The lasers used in this study were:

a) Gallium-arsenide (Ga-As) laser (**Ruđer Bošković** Institut, Zagreb, Croatia) with 904 nm pulsed beam, frequency 100—500 Hz, beam diameter 4 mm and maximal laser power 7 W.

b) Odontologic Ga-As laser D-36 (Tecno gaz, Parma, Italy), with beam diameter 3.5 mm, laser power 20 W pulsed, medium power 6 mW at emission angle of 10°, and wavelength of 904 nm. Three treatment modes, **Bio** for biostimulating use, **Pain** for antalgic use and **Push-button** for continuous emission, are available. In this study, the Bio mode was evaluated.

Both lasers emitted in the infrared (invisible) spectral region.

Preparation of bacteria

The clinically isolated oral bacterium, *Streptococcus mutans*, obtained from the Department of Microbiology, School of Medicine, University of Zagreb, was used in the study. The microorganism was grown in brain-hear lobet infusion (BHI, Difco Inc., Detroit, Mi) at 37 °C in a 5% carbon dioxide atmosphere in a static culture. Then it was maintained and characterized on tryptose blood agar plate (Difco) with 5% sheep blood prior to the experiment. Experimental culture was prepared from a 24-h culture of 10% inoculum of *S. mutans* grown in Tod-Hewitt broth (Difco Inc., Detroit, Mi). According to the turbidity of liquid cultures recorded on a Spectronic 21 spectrophotometer (Bausch & Lomb Inc., Rochester NY), the liquid suspension contained approximately 2 x 10^8 colony-forming units *per* milliliter. Using the microbiologic probe, the bacteria were maintained on 16 solid blood agar culture plates. The inoculum was dispersed in three directions. Immediately after that, the centers of 8 plates were subjected to a laser power of 7 W (500 Hz) for 3 min. The laser probe was fixed perpendicular to the plate at a 5-mm distance from the target surface. The remaining 8 plates served as controls. After the laser treatment, the plates were incubated for 24 h at 37 °C before the next session. During a six-day period, the bacteria were each day exposed at the same area. After that period, the bacteria from the target area and control plates were diluted, mounted on new plates and incubated for 24 h before scoring the growth.

Temperature measurement

Temperature measurements were performed in five healthy male subjects. The measurement sites were as follows: the tongue tip mucosa, finger pad skin, and ear lobe surface. The previous pilot study showed the tongue temperature to decrease when mouth was left open, whereas the skin areas mentioned above had a relatively constant temperature.

Temperature changes were monitored and recorded by a Topscan 808 thermovision camera (Iskra Elektrooptika, Ljubljana, Slovenia). During recording, each measurement site in the field of observation was scanned 10 times *per* second and results were stored on a VCR in real time. The system temperature sensitivity was 0.15 °C. The measurements were performed at room temperature (23 °C) and 60% humidity.

During the screening process, the head of the subject was fixed in one position. The target area was scanned with the thermovision camera for three minutes and temperature changes were recorded on a VCR tape. These results served as controls. Then the measured areas were treated with the Ga-As laser for 3 min. Temperature changes were measured for 1 min after laser treatment. The measuring results were analyzed using a TIPS 808 computerized thermal image processing system developed at the VAMS Laboratory (Visual Analysis and Measurement System, Zagreb, Croatia).

Results

During and after the six-day period in *in vitro* conditions, no bacteriostatic or bactericidal effects on *Streptococcus mutans* after Ga-As laser treatment were observed.

Temperature images of the tongue surface before and after 3-min laser treatment are presented in Figures 1 and 2. The starting temperature of the mucosa before laser session was 36.62 ± 0.258 °C. It can be noticed that the tongue temperature decreased during and after laser treatment, and was 35.68 ± 0.526 °C at the end of the measurement period. Similar results were also obtained during control screening. Temperature changes recorded on the finger pad skin and ear lobe of 36.04 ± 0.260 °C and 35.92 ± 0.248°C, did not differ significantly from those recorded during the control measurement period before laser treatment, i.e. 36.24 ± 0.343 °C and 35.92 ± 0.334 °C, respectively.

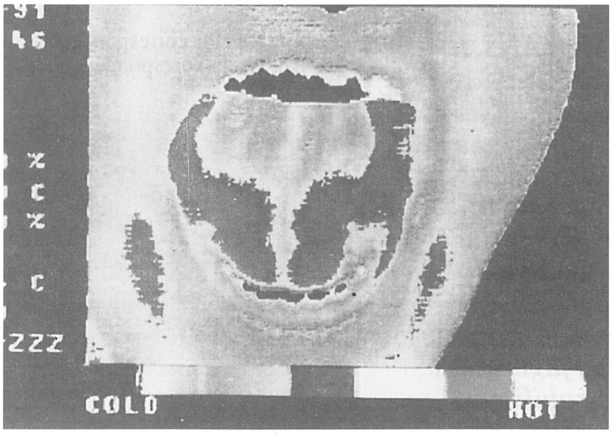

Figure 1. *Temperature images of the open mouth. Dark areas represent surface of the teeth, Y-like gray shape (arrows) at the* dorsum *of the tongue represent warmer area beneath the* arteria lingualis.

Slika 1. *Termovizijska slika otvorenih usta. Tamna područja prikazuju relativno hladne površine zuba. Obostrano izražena siva (Y-oblik) područja (strjelice) označavaju toplija područja ispod arterija lingualis na dorzumu jezika.*

Figure 2. *Temperature images of the face and tongue surface before laser treatment. Note a relatively cold surface of the nose and tip of the tongue.*

Slika 2. *Termovizijska slika raspodjele topline na licu i površini jezika. Vrh nosa i jezika hladniji su od okolnih struktura.*

Figure 3. *Temperature image of the face and tongue surface after 3-min treatment with Ga-As laser. The temperature of the tip of the tongue decreased and a larger cold area is seen.*

Slika 3. *Temperaturna slika lica i jezika nakon trominutna zračenja Ga-As laserom. Na vrhu jezika vidljivo je proširenje hladne površine.*

Discussion

In this study, the clinically isolated bacteria were used, since bacteria isolated from a particular ecological niche (possessing a cell envelope with maximal protection for the cell) are no more resistant to laser radiation than bacteria maintained in a culture collection (12). While the effects of laser on bacteria have been described in the literature, there is a controversy on the lethality of laser radiation. Obviously, the use of high laser energy (hard lasers) radiation has a lethal effect on oral bacteria (12—14). In this study, however, the soft Ga-As laser did not show such an effect on *Streptococcus mutans in vitro*. At wavelengths of less than about 450 nm, the effects are predominantly photochemical, thus chemical changes may be directly produced by the laser beam. The bacterial effect of ultraviolet light has already been demonstrated. To induce the formation of reactive species, such as singlet oxygen, superoxide ions and hydroxyl radicals, which can damage the cell (6), the energy transfer of the visible light should be provided by adding a colored compound. Such a compound can sensitize them to the visible light. However, the Ga-As laser emits wavelengths of 904 nm in the invisible part of the electromagnetic spectrum, which may additionally complicate the problem. Burns et al. (16) have reported that energy doses of the GaAlAs laser (660 nm) are much higher than those required by the He-Ne (633 nm) laser to kill similar numbers of bacteria.

At a wavelength longer than 450 nm, thermal effects are predominant. However, in case of Ga-As low-laser it seems that power wavelength is longer than that likely to produce a direct photochemical effect, and the output power is too low to cause any thermal effects (15). The results of this study are in agreement with this report. The temperature obtained at the measurement sites on the ear lobe and finger pad skin and on the tongue mucosa, even after three minutes of laser treatment, did not differ from the control values. On the tip of the tongue, a temperature decrease was observed during both control and laser screening, which could be explained by natural air circulation arround the tip of the tongue when it is outside the mouth.

Conclusion

In this study, the low-energy Ga-As laser (so-called soft laser) did not show any bacteriostatic or bactericidal effect on *Streptococcus mutans* in *in vitro* conditions. It did not induce any thermal effect on the finger pad and ear lobe skin and tongue mucosa in *in vivo* conditions either.

UČINAK Ga-As LASERA NA STREPTOCOCCUS MUTANS I TEMPERATURNE PROMJENE NA KOŽI I SLUZNICI

Sažetak

U radu je ispitivan in vitro antibakterijski učinak Ga-As niskoenergetskog lasera na oralni Streptococcus mutans. Bakterije nasađene na ploče krvnog agara obasjavane su tri minute svakih 24 sata kroz šest dana. Nakon toga, bakterije s ciljanih mjesta presađene su na nove ploče. Osim toga, na pet ispitanika ispitivan je i temperaturni učinak lasera na kožu ušne resice, jagodice prsta i sluznicu vrha jezika. U in vitro uvjetima nije zabilježen baktericidni niti bakteriostatski učinak lasera. Temperaturne promjene na koži i sluznici jezika, tijekom i nakon zračenja, nisu bile statistički vjerodostojno različite od kontrolnih vrijednosti.

Ključne riječi: *antibakterijski učinak, temperatura, laser*

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