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Inhibitory effects of a super pulsed carbon dioxide laser at low energy density on periodontopathic bacteria and lipopolysaccharide *in vitro*

Taro Kojima[1], Koichi Shimada[1,2], Hiroyasu Iwasaki[1], Koichi Ito[1,2]
[1]Department of Periodontology, [2]Division of Advanced Dental Treatment, Dental Research Center, Nihon University School of Dentistry, Tokyo, Japan

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Objective and background: Previous studies have described the effect of irradiation by a carbon dioxide (CO_2) laser at high energy density on oral bacteria, and various side-effects have also been observed. However, no published studies have examined the effect of irradiation by a CO_2 laser at low energy density on oral bacteria. The purpose of this study was to investigate the effects of super pulsed CO_2 laser irradiation on periodontopathic bacteria and lipopolysaccharide (LPS).

Methods: Bacterial suspensions of two species of periodontopathic bacteria received laser irradiation at energy densities of 0–12.5 J/cm^2. The suspensions were then spread over agar plates and incubated anaerobically. The bactericidal effects were evaluated based on colony formation. Samples of LPS were laser-irradiated at energy densities of 0–12.5 J/cm^2. The biological activity was measured, and LPS was analyzed by sodium dodecyl sulfate–polyacrylamide gel electrophoresis (SDS–PAGE).

Results: The irradiation at low energy densities of 7.5 and 12.5 J/cm^2 killed more than 99.9 and 99.999% of *Porphyromonas gingivalis* and more than 99% of *Actinobacillus actinomycetemcomitans* was sterilized by the irradiation at 7.5 J/cm^2. LPS biological activity was significantly decreased by laser irradiation at energy densities of more than 7.5 J/cm^2 ($p < 0.05$), and the components of LPS analyzed by SDS–PAGE was diminished non-specifically.

Conclusion: The results indicate that CO_2 laser irradiation at low power is capable of bactericidal effect on periodontopathic bacteria and decreasing LPS activity.

Koichi Shimada, DDS, PhD, Department of Periodontology & Division of Advanced Dental Treatment, Dental Research Center, Nihon University School of Dentistry, 1-8-13 Kanda-Surugadai Chiyoda-ku, Tokyo 101–8310, Japan
e-mail: shimada-ki@dent.nihon-u.ac.jp
Tel: +81 3 3219 8107
Fax: +81 3 3219 8349

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Periodontopathic bacteria and lipopolysaccharide (LPS) in dental plaque are involved in the initiation of gingivitis and the progression of periodontitis (1). Plaque control, the process by which plaque is removed from both supragingival and subgingival tooth surfaces, is of great importance in the prevention of periodontal diseases and can reduce levels of periodontopathic bacteria and LPS. Subgingival plaque control is more complex and difficult than supragingival plaque control because the shapes of periodontal pockets,

furcation areas, and developmental depressions are complex (2).

Previous studies (3–5) have reported that laser irradiation inhibits plaque and have concluded that lasers may be useful for plaque suppression or eradication. The effect of the carbon dioxide (CO_2) laser irradiation at high energy density on plaque control has been examined (6, 7). The high energy CO_2 laser, which operates at wavelengths between 9.3 and 10.6 μm, has been used in dentistry primarily for soft tissue procedures (8, 9), dental hard tissue procedures (10–12), and the removal of calculus (13, 14). Early studies (15–17) used continuous-wave CO_2 lasers, but these lasers have been shown to cause extensive thermal damage including carbonization of both tooth fissures and soft tissues (12, 18–20). The use of a pulsed CO_2 laser may decrease thermal damage (18, 20). It has been reported (21, 22) that dental pulp irradiated with a super pulsed laser for a short time showed temperature increases within physiologically acceptable limits, suggesting that the super pulsed laser does not cause thermal damage. Therefore, we hypothesized that irradiation by a super pulsed CO_2 laser at lower energy density would have few adverse effects on dental hard tissue and could safely be used in the subgingival area. The goal of this study was to determine the effects of super pulsed CO_2 laser irradiation at very low energy density on periodontopathic bacteria and LPS *in vitro* in order to assess the laser's usefulness as a method of plaque control in the subgingival area.

Material and methods

Laser device

A CO_2 laser device (Panalas C10, Panasonic Dental Co., Tokyo, Japan) was used at 10.6 μm wavelength. This laser model has a super pulse mode with pulses of 0.6 ms duration at intervals of 6 ms and energy densities between 0 and 5 W. The laser beam was delivered through an articulated arm with a tapered and defocused tip Type 4A (Panasonic Dental Co., Fig. 1) of diameter 5.0 mm located at the top of the arm.

Bacteria and culture

The test bacteria, *Porphyromonas gingivalis* 381 and *Actinobacillus actinomycetemcomitans* ATCC33384, were a gift from the Department of Bacteriology, Nihon University School of Dentistry. Brain heart infusion broth (Difco Laboratories, Detroit, MI, USA) with 0.5% yeast extract (Nissui Pharmaceutical Co., Tokyo, Japan) was used as the culture medium. Cultures were incubated anaerobically (N_2, 80%; H_2, 10%; CO_2, 10%) at 37°C. All bacteria were cultured to the logarithmic phase before being used in the tests.

Bactericidal effects of the CO_2 laser

Logarithmic phase test organisms were centrifuged at 3000 *g* for 15 min, and the supernatant was removed. The residue was washed two or three times with sterilized sodium phosphate buffer (pH 7.0), and the cells were harvested by centrifugation. Fresh sterilized buffer was added and cell suspensions (approximately 10^9 CFU/ml) were prepared. Cell suspension samples of 1.0 μl each in 1.5-ml microcentrifuge tubes were irradiated with the super pulsed CO_2 laser at 0.5 W for 0, 1, 3 and 5 s (the energy densities were 0, 2.5, 7.5, and 12.5 J/cm^2, respectively) from a distance of 17 mm and an irradiated diameter of 5.0 mm from the top of the tip at room temperature. The suspension was immediately diluted and spread over the surface of a brain heart infusion agar plate containing 0.5% yeast extract and 5% sheep blood. The plate was incubated anaerobically for 96 h at 37°C. The bactericidal effect was evaluated based on colony formation. Tests were performed in triplicate.

Effects of the CO_2 laser on lipopolysaccharide *in vitro*

Samples (1.0 μl) of 2.0 μg/ml LPS (*Escherichia coli* O111:B4, Sigma Chemical Co., St. Louis, MO, USA) were irradiated at 0.5 W for 0, 1, 3 and 5 s (the energy densities were 0, 2.5, 7.5, and 12.5 J/cm^2, respectively) under the conditions described above. After treatment, the biological activity of the LPS was measured by Toxicolor® (Seikagaku Kogyo Co., Tokyo, Japan) (23). This test involves factor G-free *Limulus* amoebocyte lysate and a chromogenic

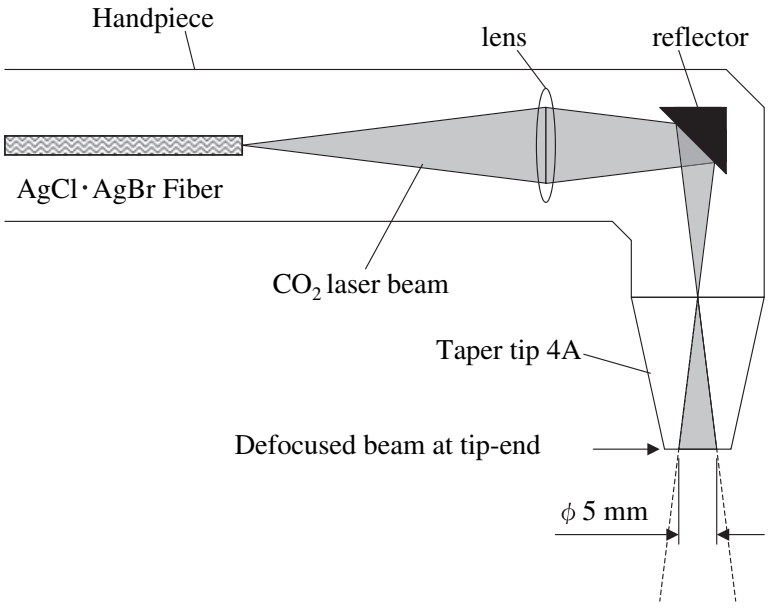

Fig. 1. Schematic figure of Type 4A tip. The tip was tapered and defocused type. The diameter was 5.0 mm.

substrate, *t*-butyloxycarbonyl-Leu-Gly-Arg-*p*-nitroanilide (LAL + *p*NA). Tubes, pipettes, and 96-well plates (Seikagaku Kogyo Co.) were endotoxin-free. A diluted sample (50 µl) and 50 µl of LAL + *p*NA were incubated at 37°C for 30 min. The absorbance of the sample following diazotization was measured at 545–620 nm. All tests were repeated five times.

Following the irradiation of 2.5 mg LPS (*E. coli* O111:B4) under the same conditions, a sample containing 200 µg of protein was analyzed by sodium dodecyl sulfate–polyacrylamide gel electrophoresis (SDS–PAGE). The gels were then stained with Coomassie Brilliant Blue R-250 (Bio-Rad Laboratories, Hercules, CA, USA).

Statistical analysis

All differences were analyzed statistically using one-way factorial analysis of variance with the Dunett's method. Statistical significance was accepted at $p < 0.05$.

Results

Bactericidal effect of the CO_2 laser

Bactericidal effects were evident following CO_2 laser irradiation of *P. gingivalis* and *A. actinomycetemcomitans* (Table 1). The irradiation at energy densities of 7.5 and 12.5 J/cm^2 killed 99.9 and more than 99.999% of *P. gingivalis*, respectively, and more than 99% of *A. actinomycetemcomitans* was killed by energy densities of more than 7.5 J/cm^2. The numbers of colony-forming units of periodontopathic bacteria irradiated at energy densities of 7.5 J/cm^2 or greater were statistically different from the control, which was not irradiated ($p < 0.0001$).

Effects of the CO_2 laser on lipopolysaccharide

Laser irradiation at energy densities of 7.5 and 12.5 J/cm^2 significantly decreased LPS biological activity compared with that of a no-irradiation group, as shown in Fig. 2 ($p < 0.05$). LPS biological activity after irradiation at 12.5 J/cm^2 was approximately 22% of that of a no-irradiation control.

Laser irradiation resulted in the diminished presence of all bands of LPS analyzed by SDS–PAGE, with the highest energy densities causing the greatest reduction in the bands (Fig. 3). However, all bands diminished by a similar amount at each energy density level.

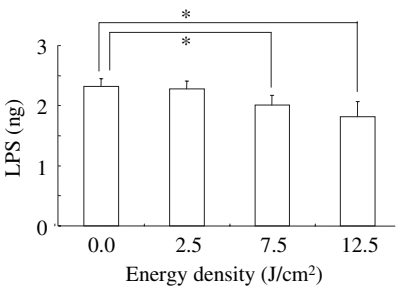

Fig. 2. The effect of irradiation by a CO_2 laser on LPS biological activity. After samples were irradiated at energy densities of 0, 2.5, 7.5, and 12.5 J/cm^2, the LPS biological activity was measured using Toxicolor®. Laser irradiation at 7.5 and 12.5 J/cm^2 significantly ($p < 0.05$) decreased LPS activity compared with that of a no-irradiation control group.

Discussion

We evaluated whether irradiation by a CO_2 laser at low energy density had a bactericidal effect on periodontopathic bacteria and an inhibitory effect on LPS. There have been no previous reports on these topics.

Previously, Dederich *et al.* (24) reported the bactericidal effects of CO_2 laser irradiation on cariogenic bacteria such as *Streptococcus* species and *Actinomyces* species *in vitro*. Their results indicated that laser irradiation at 159 J/cm^2 killed more than 99% of the test bacteria. The results of these and our studies, considered together, indicate that CO_2 laser irradiation has bactericidal effects on both periodontopathic and cariogenic bacteria; the differences in the effectiveness of laser irradiation in the two studies may be attributable to the use of different laser devices (super pulsed vs. pulsed), the proximity of the laser to the bacteria (17 vs. 300 mm), or the type of bacteria tested (periodontopathic vs. cariogenic). Periodontopathic bacteria might have a higher sensitivity to laser irradiation than cariogenic bacteria do.

The biological activity of LPS after irradiation by the CO_2 laser at an energy density of 12.5 J/cm^2 was approximately 22% of that seen in a control no-irradiation group (Fig. 2). As Fig. 3 shows, most bands of LPS were diminished by CO_2 laser irradiation. The inhibitory effects of CO_2 laser irradiation on LPS have not been reported previously, although Yamaguchi *et al.* (25) reported that erbium (Er):YAG laser irradiation diminished LPS on the root surface. Using an Er:YAG laser with a wavelength of 2.94 µm, similar to that of the peak of the infrared spectrum of LPS (2.92 µm), and an energy density of 35.4 mJ/cm^2, they reported an 83.1% reduction in LPS on the root surface. However, Er:YAG laser irradiation at 35.4 mJ/cm^2 formed craters and rough surfaces on the root dentin surfaces. The Er:YAG laser has a water absorption characteristic approximately 15 times that of the CO_2 laser (26, 27) and thus it is effective at cutting a variety of tissues with minimal collateral heat damage. As the aim of

Table 1. Bactericidal effects of CO_2 laser on periodontopathic bacteria

Energy density (J/cm^2)	*P. gingivalis*	*A. actinomycetemcomitans*
0.0	$(1.0 \pm 0.1) \times 10^6$	$(1.2 \pm 0.3) \times 10^6$
2.5	$(1.0 \pm 0.1) \times 10^6$	$(8.3 \pm 0.7) \times 10^5$
7.5	$(2.2 \pm 0.6) \times 10^2$*	$(8.3 \pm 0.9) \times 10^4$*
12.5	< 10*	$(5.8 \pm 0.6) \times 10^4$*

Mean ± SD; colony forming unit.
*One-way factorial ANOVA with Dunett's method, statistically difference compared with control (0.0 J/cm^2) ($p < 0.0001$).

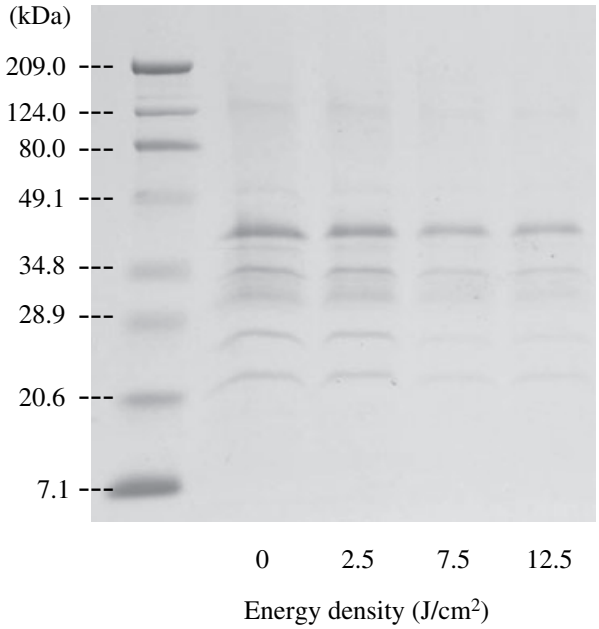

Fig. 3. The effect of irradiation by a CO_2 laser on LPS analyzed by SDS–PAGE. Following irradiation at energy densities of 0–12.5 J/cm^2, LPS was analyzed by SDS–PAGE. The gel was stained with Coomassie Brilliant Blue R-250. Laser irradiation resulted in the diminished presence of all bands, with greater effects at higher energy densities.

using the laser is to decrease LPS without damaging the root surface, the use of the CO_2 laser would be preferable to the use of the Er:YAG laser. In this study, we used a super pulsed CO_2 laser at very low energy density (less than 12.5 J/cm^2). Previous studies (22, 28, 29) have reported that irradiation with a CO_2 laser at very low energy did not change the surface morphology of enamel, dentin, or bone and did not influence the temperatures of pulp and the dentin surface, suggesting that irradiation with a CO_2 laser at very low energy density might decrease periodontopathic bacteria and LPS without damaging the hard tissues adjacent to the subgingival area.

In conclusion, the results indicate that irradiation by a super pulsed CO_2 laser at low energy density has bactericidal effects on periodontopathic bacteria such as *P. gingivalis* and *A. actinomycetemcomitans* and significantly decreases LPS biological activity. However, when the irradiation of CO_2 laser was used as plaque control, the energy might be absorbed by the dental plaque, which was biofilm. The results of this study suggest that the irradiation of the laser at an even lower energy density after the energy is absorbed by biofilm might have bactericidal effects, although further studies about the effect of the laser on biofilm is needed.

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