Effect of Galium Arsenide Diode Laser on Human Periodontal Disease: A Microbiological and Clinical Study

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Background and Objective: The present study is aimed to describe short-term results on selected microbiological and clinical parameters obtained by treatment with soft laser in conjunction with methylene blue and/or mechanical subgingival debridement in human periodontal disease.

Study Design/Materials and Methods: Ten patients, in whom each dental quadrant was randomly designated to receive one of four types of treatment procedures, were included in the study. Groups of quadrants received: scaling/root planing (SRP); laser application (L); SRP combined with L (SRP/L); oral hygiene instructions (OHI). Four single rooted teeth (one in each quadrant), having an interproximal site with a probing depth of 4 mm mesio-buccally, were selected in each patient. The selected teeth were first assessed for microbiological (one site/tooth) and then for clinical variables (six sites/tooth). Supragingival irrigation with methylene blue was performed prior to laser application. The microbiological (proportions of obligate anaerobes) and clinical measurements (plaque and gingival indices, bleeding on probing, probing pocket depth) were evaluated over a period of 32 days.

Results: Only the SRP/L and SRP groups provided significant reductions in the proportions of obligate anaerobes before and after treatments with no significant differences in between. Parallel to the microbiological changes, both SRP/L and SRP resulted in similar clinical improvements, whereas L alone revealed a limited effect similar to OHI.


Key words: anaerob micro-organisms; mechanical periodontal therapy; soft laser

INTRODUCTION

Successful periodontal treatment is dependent on the stoppage of tissue destruction, elimination or control of etiological agents together with a microbial shift toward one typically present in health [1,2]. The elimination of the pathogenic subgingival microbiota may be achieved by non-surgical scaling and root-planing [3–5]. However, mechanical therapy alone, may fail to eliminate the pathogenic bacteria because of their location within the gingival and dental tissues or in other areas inaccessible to periodontal instruments [6,7]. These limitations and the improved biological understanding of periodontal diseases together with the emerging evidence of bacterial specificity have led to a move in emphasis from a pure mechanical approach to other methods which include the use of adjunctive antimicrobial measures. Methods of killing periodontal pathogens, therefore, are of great interest and considerable attention has been devoted to the possibility of using antibiotics or antiseptics in this respect. More recently, it has been suggested that high-power lasers, such as Nd/YAG laser, which emit light in the infrared region may be useful for destroying such organisms, presumably by a thermal effect [8]. However, the clinical use of such high-power lasers introduces problems from the point of view thermal side effects on surrounding tissues [9]. An alternative approach using light in the visible region of the electromagnetic spectrum would be more attractive from the point of view safety. Although most species of oral bacteria do not absorb visible light and so are largely unaffected by such radiation, assimilation or adsorption of a colored compound by these organisms can sensitize them to visible light [10]. It has been shown in in vitro studies that it is possible to kill oral bacteria with light from a low power laser, once they have been sensitized by various dyes such as toluidine blue O or methylene blue [10–16]. This implies that low power lasers, in conjunction with appropriate photosensitizers, may be a useful adjunct to mechanical debridement in the treatment of inflammatory periodontal diseases if a similar effectiveness can be achieved in vivo. To the best of our knowledge, no investigations evaluating the use of low power soft lasers in conjunction with topically applied photosensitizers in the treatment of periodontal diseases are available in the literature. Therefore, the purpose of this study was to examine the short-term effect of low power
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soft laser therapy in conjunction with topical methylene blue and/or mechanical subgingival debridement on periodontal pockets with regard to the antimicrobial abilities and the improvement of periodontal condition.

MATERIALS AND METHODS

The study group comprised ten systemically healthy subjects with early to mild periodontitis who applied for treatment to the clinics of the Department of Periodontology at the Faculty of Dentistry, Marmara University and Yeditepe University. Patients who have taken antibiotics or received periodontal treatment within 6 months preceding the study were not included. They were instructed about the nature and purpose of the study and consents were obtained. Prior to any treatment procedure, oral hygiene instructions (OHI) were given. Each quadrant of the subjects was randomly assigned to one of the following groups: scaling and root planning combined with laser application (SRP/L), laser application (L) alone, scaling and root planning (SRP) alone, and OHI alone. Patients were asked to rinse with methylene blue (Buco bleu 15 g, Koz İlacı San. Ve Tic. A.Ş.) for 1 minutes prior to scaling and root planning (SRP) alone, and OHI alone.

The study design is presented in Table 1. Seven days after commencement of the experimental procedures, oral hygiene instructions were given. The day when microbiological samples and clinical records were taken was designated as the day 0. On the days 1 and 7, the mechanical subgingival debridement was undertaken using the ultrasonic and hand instruments for the SRP/L and SRP groups. This procedure was followed immediately by soft laser application for the SRP/L group as well as the L group. On the days 2, 4, 9, and 11, the soft laser was applied to the SRP/L and L groups. Methylene blue was applied as a mouth rinse prior to laser application. The OHI group received neither mechanical debridement nor laser application. Three weeks after therapy procedures, microbiological samples were obtained and clinical measurements were performed by a single examiner, whereas microbial culturing was done by another individual.

Site Selection

Four single-rooted teeth (one in each quadrant) having an approximal site with a probing depth of 4 mm mesio-buccally were selected in each patient. To enhance the accuracy of measurement and simplify microbial sampling, mesio-buccal sites were chosen. The selected teeth were first assessed for microbiological (one site/tooth) and then for clinical variables (six sites/tooth). Clinical measurements were performed by a single examiner, whereas microbial culturing was done by another individual.

Microbiological Procedures

After superficial cleaning of the sites with cotton pellets and drying of the supragingival area with a stream of air, samples were taken by sterile paper points inserted into the depth of the pocket, left for 10 seconds and cultured as described by Noyan et al. [17] and Kuru et al. [18]. Briefly, each sample was aseptically transferred to 4.5 ml of phosphate buffered saline (PBS) and immediately dispersed using a vortex mixer at maximal setting for 60 seconds. The dispersed samples were serially diluted, and 0.2 ml portion of 10⁻¹, 10⁻², . . . , 10⁻⁵ dilutions were spread on a solid agar medium using sterile bent glass rods.

Trypticase soy agar plate (Oxoid Ltd.; Hamsphire, England) enriched with 0.0005% hemin (Sigma Chemical Co.; St. Louis, MO, USA), 0.00005% menadione (Sigma), and 5% defibrinated sheep blood, was inoculated for non-selective bacterial growth [19]. Furthermore, trypticase soy agar plate enriched with 5% defibrinated sheep blood was used for cultivation for facultative anaerobic microorganisms.

After 7 days of incubation of the supplemented trypticase soy agar plates in Gas Pak jars (Gas generating kit, Oxoid) in an atmosphere of 95% H₂ and 5% CO₂ at 37°C, the total viable count (TVC) was determined from the dilution giving 30–300 colonies. TVC was expressed in terms of milliliter of transport medium. Colonies were identified by the analysis of colony morphology, aerotolerance, pigmentation, Gram staining procedures, motility, catalase and oxidase activity, and using API 20 A strips (BioMérieux, France). After 5 days of incubation of trypticase soy agar plate in air and 10% CO₂ at 37°C, the total number of facultative anaerobes was determined.

All the microbiologic data were transformed into colony forming units/milliliter (CFU/ml). Obligate anaerobic bacteria was calculated as the total counts of anaerobically cultivable bacteria (TVC) minus the total counts of facultatively anaerobic bacteria and expressed as a percentage of TVC.

Clinical Parameters

Clinical measurements were performed at the selected teeth that were assessed for microbiological variables. The measurements included plaque index (PI) [20], gingival index (GI) [21], bleeding on probing (BOP), and probing pocket depth (PPD) to the nearest mm using a calibrated manual probe (PQ-OW Chicago, IL, USA, Hu-Friedy Instrument Co.).

Laser

The laser used was a Gallium-Arsenide diode laser (BTL-2000 Prague, Check, Rep., BTL Co., Check Rep.) operating at a frequency of 5.0 Hz and delivering a 30 mW continuous wave output at 685 nm with a power density of 1.6 J/cm². Patients received 1.11 minutes treatment three times a week over each papillary region as recommended by the manufacturer. During application, protective eyeglasses were worn both by the operator and the patient.

Study Design

The study design is presented in Table 1. Seven days before commencement of the experimental procedures, oral hygiene instructions were given. The day when microbiological samples and clinical records were taken was designated as the day 0. On the days 1 and 7, the mechanical subgingival debridement was undertaken using the ultrasonic and hand instruments for the SRP/L and SRP groups. This procedure was followed immediately by soft laser application for the SRP/L group as well as the L group. On the days 2, 4, 9, and 11, the soft laser was applied to the SRP/L and L groups. Methylene blue was applied as a mouth rinse prior to laser application. The OHI group received neither mechanical debridement nor laser application. Three weeks after therapy procedures, microbiological samples were obtained and clinical measurements were repeated.

After completion of the experimental period, the quadrants which received laser application alone and OHI alone were subjected to further mechanical subgingival debridement.

Statistics

Differences between the pre- and post-treatment values within each group and differences between the changes of
the pre- and post-treatment values among groups were compared using the Wilcoxon matched-pairs signed rank test [22] using the NCSS statistics package program on an IBM compatible computer. The probability value for statistical significance was set at \( P < 0.05 \).

**RESULTS**

There were no complaints such as discomfort, sensitivity or pain from subjects immediately after laser irradiation as well as 3 weeks post-therapy. The approach of patients appeared to be positive toward laser.

**Microbiological Assessments**

TVC (total anaerobically grown) and obligate anaerobic micro-organisms (total viable counts of anaerobically cultivable bacteria minus the total counts of facultatively anaerobic bacteria determined using parallel sets of aerobically and anaerobically incubated agar plates) expressed as a percentage of TVC in subgingival samples before and after different treatments, are given in Tables 2 and 3.

Following subgingival mechanical debridement combined with laser application (SRP/L), a decrease in TVC from the mean baseline value of 19.08 ± 18.62 to 15.31 ± 20.67 was noted. However, this reduction along with minor fluctuations in other groups was not significant (Table 2).

Table 3 demonstrates the differences from baseline in percent obligate anaerobes of TVC in four test groups. The proportions of obligate anaerobes decreased notably in all groups. However, only the SRP/L and SRP groups provided significant changes from baseline to 32 days post-therapy values (from 50.54 ± 27.29 to 16.36 ± 22.28, and from 47.66 ± 26.62 to 16.06 ± 17.54, respectively) \((P < 0.05)\). When changes in the proportions of obligate anaerobes between the four groups were compared, as shown in Table 4, the differences between the SRP/L and L, SRP/L and OHI, and SRP and OHI were found to be significant \((P < 0.05)\).

**Clinical Assessments**

Improvements with respect to clinical parameters occurred in all groups between the baseline and post-therapy measurements. The analysis of the PI (Fig. 1) and GI (Fig. 2) parameters indicated significant reductions from baseline to day 32 for all groups \((P < 0.05)\). With respect to the BOP, significant reductions were observed in the SRP/L and SRP groups \((P < 0.05)\), whereas the reductions in this parameter of the L and OHI groups were found insignificant (Fig. 3). Similarly, PPD declined significantly in the SRP/L and SRP groups after treatment

<table>
<thead>
<tr>
<th>TABLE 1. Study Design</th>
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<tbody>
<tr>
<td>Procedure</td>
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<tr>
<td>Oral hygiene instructions</td>
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<tr>
<td>Microbiological sampling</td>
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<tr>
<td>Clinical measurements</td>
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<tr>
<td>Mechanical debridement</td>
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<tr>
<td>Laser application</td>
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<td>Laser application</td>
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<td>Laser application</td>
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<tr>
<td>Mechanical debridement</td>
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<td>Laser application</td>
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<td>Laser application</td>
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<tr>
<td>Laser application</td>
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<tr>
<td>Microbiological sampling</td>
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<tr>
<td>Clinical measurements</td>
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</table>

**TABLE 2. Total Viable Counts (×10^3 CFU/ml) of Subgingival Samples at the Baseline and 3 Weeks After Treatment**

<table>
<thead>
<tr>
<th></th>
<th>SRP and laser</th>
<th>Laser</th>
<th>SRP</th>
<th>OHI</th>
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</thead>
<tbody>
<tr>
<td>Baseline</td>
<td>19.08 (± 18.62)</td>
<td>15.69 (± 8.92)</td>
<td>10.57 (± 7.19)</td>
<td>12.60 (± 8.32)</td>
</tr>
<tr>
<td>Post-therapy</td>
<td>15.31 (± 20.67)</td>
<td>15.89 (± 9.40)</td>
<td>8.41 (± 7.40)</td>
<td>11.04 (± 8.36)</td>
</tr>
<tr>
<td>Z</td>
<td>1.27 NS</td>
<td>0.05 NS</td>
<td>0.56 NS</td>
<td>0.36 NS</td>
</tr>
<tr>
<td>P</td>
<td>0.20 NS</td>
<td>0.96 NS</td>
<td>0.58 NS</td>
<td>0.72 NS</td>
</tr>
</tbody>
</table>

Data are presented as the mean values and the numbers in brackets are the standard deviations.

SRP, scaling and root planning; OHI, oral hygiene instruction; NS, not significant.
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TABLE 3. Proportions of Obligate Anaerobes Expressed as Percentage of Total Viable Counts at the Baseline and 3 Weeks Post-Therapy, and the Change (Baseline—3 Weeks)

<table>
<thead>
<tr>
<th></th>
<th>Laser and SRP</th>
<th>Laser</th>
<th>SRP</th>
<th>OHI</th>
</tr>
</thead>
<tbody>
<tr>
<td>Baseline</td>
<td>50.54 (±27.29)</td>
<td>52.42 (±23.10)</td>
<td>47.66 (±26.62)</td>
<td>37.19 (±26.41)</td>
</tr>
<tr>
<td>Post-therapy</td>
<td>16.36 (±22.28)</td>
<td>41.59 (±24.13)</td>
<td>16.06 (±17.54)</td>
<td>27.04 (±23.18)</td>
</tr>
<tr>
<td>Change</td>
<td>34.18* (±29.58)</td>
<td>10.88 (±29.75)</td>
<td>31.59* (±28.35)</td>
<td>10.15 (±18.36)</td>
</tr>
</tbody>
</table>

Intergroup Statistics

T = 8.49
P = 0.037**

*P < 0.05, intragroup comparison.
**P < 0.05, intergroup comparison.
SRP, scaling and root planing; OHI, oral hygiene instruction.

procedures (P < 0.05), as shown in Figure 4. Although the PPD score also tended to decrease in the L and OHI groups, these reductions were not significant.

Comparisons of the changes in the clinical parameters before and after therapy among the groups are shown in Table 5. Similar changes were observed in the SRP/L and SRP groups and the differences between these two groups in all clinical parameters were not statistically significant. In contrast, the L group demonstrated significantly less reductions in the PI, BOP, and PPD measurements compared to the SRP/L group (P < 0.05) except the GI parameter. In addition, no significant differences were noted between the L and OHI groups.

Taking the changes in all microbiological and clinical parameters into consideration, the mechanical subgingival debridement alone or in combination with laser application was observed to be more effective as compared to laser application alone.

DISCUSSION

Laser technology originated in 1960 and has developed since then. Recently the use of laser therapy has appeared with increasing frequency in the dental literature. It should be emphasized that there are different theories on the effects of laser and still many questions concerning its therapeutic value are unanswered. At the present time, the antimicrobial effects of low power lasers have not been substantiated. In assessing the potential antimicrobial effects of low power laser irradiation, a number of investigations to date has been done. Moritz et al. in their studies suggested that irradiation with the diode laser with a wave length of 805 nm facilitates bacterial elimination from periodontal pockets [23,24]. On the other hand, in vitro studies pointed out that in the absence of an appropriate photosensitizer, exposure to low power laser light had no significant effect on the viability of the pure cultures of suspected periodontal pathogens such as Porphyromonas gingivalis, Actinobacillus actinomyctecomitis, and Fusobacterium nucleatum [10–12]. It is also reported that oral bacterial species most of which do not absorb visible light and so are unaffected by such irradiation can be killed by red light from a helium/neon laser following sensitization with various dyes, especially toluidine blue O and methylene blue [10–13]. Haas et al. in another in vitro study found that dye/laser treatment resulted in the destruction of bacterial cells on different implant surfaces [14]. In a recent study, Dortbudak et al. evaluated the laser effect on peri-implantitis-associated bacteria in vivo [25]. Although the complete elimination of bacteria was not achieved in this study, authors confirmed the bactericidal effect of toluidine blue O/laser treatment when the dye applied topically on implant surfaces.

Given the problems in extrapolating irradiation parameters and findings from in vitro research to human practice, trials in humans are essential. The use of dye/soft lasers in periodontal treatment in terms of their bactericidal effects has not been investigated in vivo. It is observed that higher doses are required to produce in vivo clinical effects than those commonly used for in vitro research [26]. One of the major problems in evaluating the laser efficacy is the determination of the optimal dosage and treatment schedule. With low power lasers, this remains an area of controversy both in medicine and dentistry. Although there is some guidance from other experiments, the choices remains discouragingly wide. Furthermore, there are great differences in the published literature in terms of experimental and assessment methods and irradiation conditions. The laser used for therapy in this study was a Gallium–Arsenic diode laser operating at a frequency of 5.0 Hz and delivering a 30 mW continuous wave output at 685 nm with a power density of 1.6 J/cm². Patients received 1.11 minutes treatment three times a week over each papillary region as recommended by the manufacturer. In the present well-controlled split-mouth study providing a comparison by eliminating subject-based differences, topical methylene blue/laser

TABLE 4. Intergroup Comparison of the Changes in the Proportions of Obligate Anaerobes Before and After Treatments

<table>
<thead>
<tr>
<th></th>
<th>Laser and SRP</th>
<th>Laser</th>
<th>SRP</th>
<th>OHI</th>
</tr>
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<tbody>
<tr>
<td>Laser and SRP</td>
<td>2*</td>
<td>0.53</td>
<td>2.11*</td>
<td></td>
</tr>
<tr>
<td>Laser</td>
<td>2*</td>
<td>1.81</td>
<td>0.38</td>
<td></td>
</tr>
<tr>
<td>SRP</td>
<td>0.53</td>
<td>1.81</td>
<td>2.11*</td>
<td></td>
</tr>
<tr>
<td>OHI</td>
<td>2.11*</td>
<td>0.38</td>
<td>2.11*</td>
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</table>

Data are presented as the z value.
*P < 0.05.
treatment produced no significant antimicrobial effects at the aforementioned settings.

Methylene blue was used as the photosensitizer and applied as a mouth rinse prior to laser irradiation, since it is expected that agents in mouth rinses during supragingival irrigation can be projected into pockets less than 5 mm in depth and access to subgingival plaque can be achieved [27,28]. No significant reductions in the proportions of subgingival obligate anaerobes were detected before and after laser treatment alone. Within SRP and SRP/L groups, significant reductions in the proportions of obligate anaerobes were observed before and after treatments. However, intergroup comparison revealed no significant differences in between the groups SRP and SRP/L.

Clinical results of this study showed improvements when parameters recorded at the baseline and 3 weeks after procedure were compared. All treatment groups showed decreases in the PI, GI, BOP, and PPD parameters. However, significant reductions in PPD and BOP were observed only in the groups where mechanical subgingival debridement was performed (the SRP/L and SRP groups). This is consistent with the other studies in the related literature confirming the importance of mechanical debridement as the cornerstone for control and prevention of periodontal disease [1,17,29,30]. On the contrary, laser application without elimination of local aetiological factors resulted in insignificant reductions in PPD and BOP similar to oral hygiene regimens [31,32]. Supragingival plaque removal alone is unlikely to be sufficient to control periodontal diseases as also demonstrated by Listgarten et al. [3] and Beltrami et al. [33]. However, some shrinkage of the gingival tissues with some reduction of inflammation may occur [17,32,34]. L and OHI groups seems to have the least favorable clinical results when compared to SRP/L and SRP alone. This may indicate the unfavorable healing at the base of the pocket due to the lack of any subgingival treatment.

Periodontal diseases are bacterial infections and therefore the aim of the periodontal therapy is to eliminate or control the periodontopathic bacteria. Direct subgingival delivery of methylene blue in different concentrations should be performed to further investigate the potential antimicrobial effect of soft lasers in human periodontal disease. Dosimetric factors are also of critical importance [15,35]. The essential question is whether soft laser can provide equal or improved treatment over conventional methods in terms of antimicrobial effects.

We do feel that more research is required to effectively determine optimal treatment parameters/regimens for the significance of applying a new treatment method which is low cost, not painful, apparently harmless, and technically...
TABLE 5. Comparison of Pre- and Post-Therapy Changes (Mean ± SD) in Clinical Parameters Among Four Groups

<table>
<thead>
<tr>
<th>Parameters</th>
<th>SRP and Laser</th>
<th>Laser</th>
<th>SRP</th>
<th>OHI</th>
</tr>
</thead>
<tbody>
<tr>
<td>Plaque index</td>
<td>1.60 ± 0.47</td>
<td>0.71 ± 0.36</td>
<td>1.57 ± 0.27</td>
<td>0.64 ± 0.28</td>
</tr>
<tr>
<td>Gingival index</td>
<td>1.03 ± 0.65</td>
<td>0.60 ± 0.57</td>
<td>1.17 ± 0.68</td>
<td>0.53 ± 0.42</td>
</tr>
<tr>
<td>Bleeding on probing</td>
<td>60 ± 28</td>
<td>17 ± 8</td>
<td>50 ± 25</td>
<td>20 ± 9</td>
</tr>
<tr>
<td>Probing pocket depth</td>
<td>0.66 ± 0.43</td>
<td>0.23 ± 0.18</td>
<td>0.49 ± 0.29</td>
<td>0.19 ± 0.14</td>
</tr>
</tbody>
</table>

*P < 0.05.
SRP, scaling and root planing; OHI, oral hygiene instruction.

an easy treatment to perform should not be overlooked. If the in vitro bactericidal effectiveness of dye/soft laser can be achieved in vivo, low power lasers in conjunction with photosensitizer may be useful in the treatment of inflammatory periodontal diseases.

ACKNOWLEDGMENTS

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