Original Article

Response of Gingival Tissue Irradiated by CO₂ Laser
Approaching from the Periodontal Pocket SpaceN.H.M. KHAIRUL MATIN, Naoyuki TAGUCHI,
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CO₂ レーザーを歯周ポケットに照射した際の
歯肉組織の反応について

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要旨: 感染した接合上皮と結合組織を無菌化することを目的として、歯周ポケットにCO₂ レーザーを照射した際の歯肉組織の変化について検討した。実験には、デフォーカス型のCO₂ レーザーと6頭の雑種成犬を用いた。実験に先立ち、イヌには毎日15~20分のブラッシングを1週間続けて行った。この時点での歯周ポケットは、最高で3mmであった。実験的な歯肉炎は、軟食を与えてブラッシングも中止することにより引き起こし、4週間後には平均で1~2mm歯周ポケットが増加した。小白歯と大白歯の頬側歯肉45部位に、0.3Jから2.5Jの範囲で出力を変化させて照射した。他の15部位については、照射せずにコントロールとした。照射後7日と14日に、臨床的および組織学的に歯肉組織の反応を観察した。照射直後には、軽度の歯肉炎が観察された。組織の破壊程度は出力に関係しており、すなわち最大の組織破壊(到達深度が1.4mmで創傷領域が0.33mm²)は最高出力(2.5J)の場合に起こった。7日後には、照射された歯肉の再上皮化がほぼ完全になされており、結合組織にも新生したコラーゲン線維が形成されていた。特に0.5~1.5Jの照射出力の時は、コントロールよりも健康的な歯肉が臨床的にも組織学的にも認められた。この時期には、炎症性の細胞は顕著に減少していた。14日後になると、レーザーを照射した歯肉は完全に治癒し、傷害を受けた痕跡は全く観察されなかった。今回の実験から、周囲組織を損傷せずに感染した接合上皮を無菌化することができた。

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Key words=CO₂ laser, periodontal pocket, gingival tissue**キーワード=**CO₂ レーザー, 歯周ポケット, 歯肉組織

Introduction

History of laser treatment is not very long, begun just after Maiman's invention of ruby laser in 1960¹⁾. Compared to that, laser research expanded enormously in the medical and dental fields. Different types of lasers are being used clinically, in particular lasers are being used mostly for surgical procedures. CO₂ laser, one of the leading laser type, has been used preferably in some recent surgical procedures i.e. soft tissue incisions and ablations of delicate tissue in the fields of plastic surgery, dermatology, orthopedics, ophthalmology, neurology, urology, thoracic surgery, gastrointestinal surgery, cardiovascular surgery, and as well as in recent years in dentistry²⁾. Benefits in using the CO₂ laser in surgical purposes are well demonstrated and established. Advantages of CO₂ laser surgery include minimum hemorrhage yielding a dry field, noncontact surgery, sterilization of the surgical area, prompt healing, minimal post operative discomfort, less time consumption in performing the surgery^{3,3)}. All these benefits are behind the laser's increasing popularity among the dental surgeons. The CO₂ laser has been used mostly for soft tissue surgery including gingivectomies and gingivoplasties. There are some experimental reports demonstrating curettage of inner gingiva by CO₂ laser and healing process after irradiation⁴⁾. By changing the approach, the present experiment sequentially demonstrates the macroscopic changes, histomorphometry of the laser wound and histological healing process of inner gingiva irradiated with CO₂ laser.

Curettage is a technique designed to remove, by surgical debridement, the inner aspect of the diseased gingival wall, including the ulcerated and hyperplastic pocket epithelium and continuous zone of damaged connective tissue downward and outward to the firm and intact aspect of the gingival corium⁵⁾. Removal of the pocket epithelium has been a most discussed point in the curettage technique, but there is no doubt that connective tissue must be exposed to provide the sources for repair and to provide a

foundation for reepithelization⁵⁾. Removal of sulcular epithelium was performed by instrumentation after curettage⁶⁾. It was also demonstrated that hand curettage removed the crevicular epithelium as well as the greater portion of inflamed tissue⁷⁾. In another report it was described that hand instrumentation eliminates infected connective tissue irregularly, rather, ultrasonic curettage produced smooth wound inducing quick and effective healing⁸⁾. Although, electrosurgery is another effective modality for gingival tissue management in conjunction with restorative dentistry⁹⁾, it has also got some limitations e.g. in case of peri-implantitis or in case of a patient having a pacemaker, electrosurgery is not absolutely safe. Moreover, electrosurgery produces lacerated wound and sometimes bleeding doesn't stop instantly. On the other hand, as periodontal and peri-implantal diseases are two of the major problems in dentistry, better treatment modalities have been searched for many years. In this respect, as a new surgical tool for periodontal therapy CO₂ laser also needs to be investigated from the very primary stage. Secured level of energy, application technique, healing response of gingiva and periodontium are required to be verified.

Moreover, disinfecting or sterilizing the junctional epithelium or pocket epithelium is very essential, particularly after mechanical scaling or before preparing a prosthesis. CO₂ laser would have been useful for these purposes, unfortunately at the present stage enough experimental data is not available to use CO₂ laser at the clinic with full confidence. More and more experimental and clinical data would be helpful to establish the laser as a safe and effective dental equipment. Thus, this study was undertaken to investigate the effects of CO₂ laser irradiation on the gingiva approaching from the periodontal pocket space. At the beginning, it was started with gingivitis specially where gross gingivectomies are not indicated. Therefore, the purposes of the preliminary research were 1) a feasibility test, whether gingival tissue heals spontaneously after being

irradiated by defocused type CO₂ laser and 2) search for clinically applicable and effective energy for sterilization of infected junctional epithelium and underlying connective tissue by keeping the oral epithelium intact.

Materials and Methods

A defocused type of CO₂ laser unit, NIIC15 IR204 SURGICAL LASER SYSTEM (NIIC, Tokyo, Japan), was used in this experiment. The wavelength of the laser is 10.6 μ m and a He-Ne red light helps to guide the laser. Pulsed or continuous laser can be delivered by adjusting the output from 1 W to 15 W and irradiation time from 0.1 s to 9.9 s (Fig. 1a). The hand piece of the unit is equipped with a 0.9 mm diameter tip (Fig. 1b).

In total, 60 buccal gingival sites of six mongrel dogs weighing from 7 to 10 kg were used in this experiment.

IBAS 2000 (Carl Zeiss, Kontron M14, Germany) computerized image analyzing system was used to measure penetration depth and wound area.

The dogs underwent pre-experiment oral hygiene treatment and regular 15 to 20 minutes tooth brushing. Sub-gingival scaling and plaque removal were followed by regular tooth brushing for 7 days. At this stage maximum periodontal pocket depth was 3 mm. For pre-experimental plaque accumulation and induction of gingivitis the dogs were fed with soft food and ceased all oral hygiene measures

for the next 4 weeks^{10,11)}. At this stage considerable amount of plaque was accumulated, the pocket depth increased by 1 to 2 mm, and BOP (bleeding on probing) were also present. In this condition, 45 buccal gingival sites of premolar and molar teeth were selected for irradiation and 15 others served as control. After marking 5 mm of each gingiva mesio-distally irradiation was performed by changing the energy according to the irradiation conditions shown in Fig. 2. The gingivae were irradiated by inserting the laser tip into the periodontal pockets and keeping it 1 mm above the base of the clinical pocket. One laser ignition was applied in one position without moving the tip, in this way each selected gingival site was irradiated 5 times keeping a 1 mm gap between two ignitions.

The gingival tissue destruction and healing response were observed clinically and microscopically and compared with control. Two dogs were sacrificed by perfusion fixation under general anesthesia and gingival specimen were immersed into 10% formaldehyde for 24 hours. Then, the specimens were immersed into Plank-Rychlo solution for 7 days to decalcify adjoining bone and tooth structures. Gingival biopsy including nonlased control gingivae were taken from other 4 dogs immediately, 7 days and 14 days after the irradiation. The biopsy specimens were immediately immersed into 10% formaldehyde and kept for 24 hours. All of the specimens were embedded in paraffin, 5 μ m

Fig. 1a NIIC15 IR204 SURGICAL LASER SYSTEM, a defocused type CO₂ laser unit.

Fig. 1b The hand piece of the laser unit, equipped with a 0.9 mm diameter tip.

sections were cut and mounted on glass slides and then stained with Hematoxylin-Eosin or Azan-Mallory stains. All the stained specimens

Fig. 2 Irradiation conditions of the CO₂ laser applied in this experiment.

were examined under light microscope. Histomorphometrically 1) penetration depth of the CO₂ laser into the gingival tissue and 2) gingival wound area produced by the laser were measured by the image analyzer. Histomorphometry was performed by directly inputting the images from the microscope.

Results

On clinical examination it was found that the gingiva became more reddish and inflamed due to laser ablation just after irradiation (Fig. 3b). Mild bleeding was also observed, maybe because of the injury occurred during the insertion of metal tip of the laser hand-piece rather than laser irradiation. Redness disappeared after

Fig. 3a

Fig. 3b

Fig. 3c

Fig. 3 Photograph displays the buccal gingiva of a dog molar tooth, the part of the gingiva pointed by white arrow in between the black bars is the site selected for irradiation by CO₂ laser (a) is the condition before irradiation, redness and swelling indicates inflammation of the gingiva. (b) is the condition immediately after irradiation, shows acute inflammatory condition (redness and swelling increased). (c) is the condition 7 days after irradiation, inflammatory condition disappeared (redness and swelling reduced considerably).

Fig. 4a

Fig. 4b

Fig. 4c

Fig. 4d

Fig. 4 Hematoxylin-Eosin stained gingival sections- a) is a nonlased control section shows plenty of inflammatory cells occupied the sulcular part of the gingiva. Hypervascularization and distortion of epithelial and connective tissue structure are to be noticed. b), c) and d) are the gingival sections immediately after irradiation CO₂ laser wound just after irradiation with 0.3 J, 0.9 J and 2.5 J respectively. Tissue ablation & fusion, carbonization and vacuole formation were dependently increased with the intensity of the laser.

7 days and healthy gingiva could be detected (Fig. 3c), inflammatory evidence, been present before laser irradiation (Fig. 3a) also disappeared. After 14 days the laser treated gingivae showed normal healthy appearance.

On microscopical examination the control

specimen demonstrated gingivitis with junctional epithelium completely losing it's normal structure. Plenty of inflammatory cells and dilated capillaries infiltrated the whole sulcular part of the gingiva (Fig. 4a). The laser irradiated gingivae demonstrated comet-tail shaped vacuoles

Fig. 5a

Fig. 5b

Fig. 5 Higher magnification of Hematoxylin-Eosin stained sections, a) is the control specimen, arrow showing inflammatory cell, there are plenty of inflammatory cells occupied the sulcular part of the gingiva. b) is a section taken 7 days after irradiation, inflammatory cells disappeared, epithelial and connective tissue healing is in progress.

were seen due to tissue vaporization by the laser which were marked by carbonized lining and secondarily affected tissues were fused by absorbed heat (Fig. 4b, 4c & 4d). Severity of tissue destruction varied according to energy intensity of the laser, in some cases laser didn't even penetrate the full epithelial layer and lamina propria (Fig. 4b), in some cases it penetrated deep inside the connective tissue and nearly destroyed the whole gingiva (Fig. 4d).

After 7 days newly forming epithelium took it's position, collagen fiber and blood vessels were regenerating smoothly and the inflammatory cells were outnumbered by new fibroblasts (Fig. 5b). In some cases healing was delayed due to the massive tissue destruction and due to the presence of carbon particles, specially when energy were 1.5 J or more. Whereas, the control specimen demonstrated gingivitis with distorted junctional epithelium, plenty of inflammatory cells and hypervascularization specially in the sulcular part (Fig. 5a).

Azan-Mallory stained specimen showed that no damage occurred to the adjoining bone and tooth structures immediately after laser irradiation (Fig. 6a). By 7 days, reepithelialization of the lased gingiva nearly completed to make a strong inner barrier for the gingiva, healing of underlying connective tissue continued smoothly with the formation of new collagen fiber (Fig.

6b). At 14 days lased gingivae healed completely without any trace of laser injury. Inflammatory cells also disappeared remarkably at this stage (Fig. 6c).

Histomorphometry showed that severity of tissue destruction was energy dependent, i.e. maximum tissue destruction (penetration depth = 1.4 mm and wound area = 0.33 mm²) was produced by maximum energy (2.5 J) used (Fig. 7a & 7b). Laser energy 0.5 J and 1.5 J penetrated in an average of 0.5 mm and 1.2 mm respectively which is quarter to half of the bucco-lingual thickness of the free gingiva.

Discussion

Reviewing all the results it was found that the wound produced by the vaporizing effect of the CO₂ laser on the gingiva heals reasonably well and improvement of the inflammatory aspect could also recognize. Reepithelialization of junctional epithelium is important for gingival healing to make the inner barrier which very often gets infected or distorted. Once this barrier gets distorted and untreated periodontal disease proceeds, loss of periodontal ligaments occurs, then epithelium migrates down along the root surface¹²⁾. Similar type of occurrences are also seen in case of peri-implantal gingiva. In all of those cases CO₂ laser may be useful to prevent gingivitis to proceed further without

Fig. 6a

Fig. 6b

Fig. 6c

Fig. 6 Azan-Mallory stained gingival specimen, a) is immediately after irradiation, asterisk and 'AB' indicates the tooth structure and alveolar bone respectively, no damage can be detected to the adjoining bone and tooth structures. Arrow indicates the laser ablated portion of the gingiva. b) is a section after 7 days, reepithelialization of the lased gingiva nearly completed, healing of underlying connective tissue continued smoothly with the formation of new collagen fiber. c) is a section 14 days after irradiation shows lased gingivae healed completely without any trace of laser injury. Inflammatory cells also disappeared remarkably.

elaborate surgical procedures, as demonstrated in the present experiment. Rossmann et al. stated method of CO₂ laser application on an advanced periodontal disease condition to retard epithelial

downgrowth or migration⁵⁾. The approach of present pilot study was different from Rossmann et al.'s study by means of irradiation method and histomorphological examinations.

Fig. 7a 'Penetration depth' of the CO₂ laser into the gingiva. Increased according to the intensity of the laser energy.

Clinical and histological features represented healthy gingival tissue compared to the control particularly when the irradiation energy was between 0.5 J to 1.5 J. The range of irradiation energy histomorphologically covered quarter to half of the bucco-lingual width of the gingiva indicating that this range of CO₂ laser energy is safe and effective for curetting sulcular part of the gingiva. The heat induced by the laser may have worked in several ways e.g. by burning the infected tissue directly, by dilating the adjoining vascular tissues increasing the circulation and stimulating the overall healing process. In some cases healing was delayed due to the massive tissue destruction and due to the presence of carbon particles specially when energy was 1.5 J or more. Presence of carbon particles at the wound wall may have delayed healing of the gingiva, but didn't really harm surrounding tissue or didn't induce tissue necrosis. This study may be an experimental model for the investigators those who are interested in additional experiments regarding gingival treatment by CO₂ laser. Some interesting experiments were reported defining the effects of CO₂ laser on dental hard tissues including dentin, cementum and alveolar bone^{13,14,15}. New bone formation and later changing to mature bone was observed after CO₂ laser irradiation in

Fig. 7b 'Wound area', formed almost similar curves in relation to delivered energy.

dog, even at 20 J high energy level¹⁶. In the present experiment 2.5 J was the highest laser energy which is eight times less than the above mentioned energy. The direction of laser irradiation in this experiment may be the reason that didn't injure tooth structure or periodontal tissue, low energy level also may be another reason. Unlike J. F. Barahona R.'s experiment the morphology of the laser wound was comet-tail shaped rather than triangular-shaped crater¹⁶, may be because of the low energy level irradiated from a defocused type laser unit. Thus, as long as safety is concern, energy up to 1.5 J of the NIIC 15 laser unit appeared to be dependable.

Nevertheless, CO₂ laser curettage was compared only with nontreated control in the present experiment and it was found that infected gingival tissue effectively heals after irradiation indicating only that the CO₂ laser can be used for gingival curettage. For free movement inside the periodontal pocket a flexible and smaller diameter laser tip would have produced even better result. It must be interesting to compare the conventional gingival curetting methods to curettage by CO₂ laser. More experiments on effects of CO₂ laser irradiation on dental pulp, periodontal ligament, alveolar bone and other tooth structures are expected.

Conclusion

At the conclusion the following suggestions can be made: 1) it is feasible to evaporate pathologic tissue from the sulcular part of the gingiva by CO₂ laser to sterilize the junctional epithelium and underlying connective tissue, and 2) 0.5 J to 1.5 J of the NIIC15 laser unit appeared to be the optimum energy range for gingival curettage.

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