Stability and Effectiveness of Laser and Liquiband Treatments in the Rat Model of Oral Ulcer

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Background and Objectives

This study was undertaken to assess the stability and efficacy of laser therapy (808 nm), Liquiband (a commercial topical skin adhesive product), and a combination treatment, for application in oral ulcers.

Materials and Methods

The oral ulcer rat animal model was used to determine the efficacy of photobiomodulation, Liquiband, or combination therapy. Ulcers were induced by injecting 60% acetic acid in the oral mucosa. Three days after ulcer confirmation, the chemically induced ulcers were treated with either laser (808 nm), Liquiband, or a combination of both, every two days for 8 days (4 sessions). Combination therapy was performed by first treating with laser followed by application of the Liquiband. Reduction in ulcer area size was subsequently determined, and animals were sacrificed at 5 and 10 days after the last administration, for histological observation of the extracted oral ulcer tissue samples.

Results

In this study, treatment with either laser (808 nm) or commercial Liquiband product as well as the combined treatment significantly enhanced oral ulcer healing in the rat animal model. The effect of laser treatment is mainly attributed to collagen synthesis, whereas application of the Liquiband promotes vascularization and formation of granulation tissue. Our results indicate that further optimization of the combined therapy could synergistically and significantly enhance oral ulcer healing.

Conclusion

Laser or Liquiband treatment of oral ulcer in the rat animal model exert different outcomes, but both methods confirm significant effects relative to the untreated group. The combined treatment group with laser and Liquiband showed marked oral ulcer healing, but further optimization is required to get highly significant results and establish the potential synergistic effect of the combination therapy, as compared to the individual enhancing effects of healing oral ulcers.

Key words

Oral ulcer; Low-level light therapy; Cyanoacrylate

INTRODUCTION

An oral ulcer is an inflammatory disease in the mucous membrane of the mouth caused by damage and necrosis of the surface tissues of the mouth and lips. Oral ulcers lead to severe pain, burning and increase the risk of infection through the wound.¹ The causes of oral ulcers include infection through bacteria, immune disorders, blood diseases and wound caused by foreign substances or external stimuli.^{1,2}

Aphthous stomatitis is the most common oral inflammatory disease. Many patients are suffering from recurrent nonspecific stomatitis on the oral mucosa, and the prevalence in the general population ranging between 5% and 60%.³ Recurrent aphthous stomatitis is a multifactorial disease, including genetic causes, and over 40% of patients have a family history.⁴ Characterized by recurrent oral mucosal ulceration can persist throughout the patient's lifetime, with no gender predilection.⁵

Even recently, laser treatment is used to treat oral ulcers, and it has been reported that low-power diode lasers reduce healing time and pain intensity in patients with aphthous stomatitis.^{6,7} The laser stimulates cells to promote the production of ATP in mitochondria, thereby decreasing the oxygen consumption of cells, which leads to exhibiting anti-inflammatory effects. It also reduces prostaglandins to reduce pain.⁸⁻¹⁰

Liquiband is an adhesive agent for soft tissue suturing and contains 2-octyl cyanoacrylate. cyanoacrylate was first presented in 1959, but the toxicity of the degradation products was a problem.¹¹ Afterward, a non-toxic cyanoacrylate adhesive appeared, providing a suture that is faster and less traumatic than sutures, and is used to suture skin incisions and lacerations in areas with low skin tension.¹² The microbial barrier created by the adhesive protects the wound from microbial contamination.^{13,14} The purpose of this study is to investigate the stability and effectiveness of the laser and Liquiband in an animal model of oral ulcer through histological data on the healing effect and interaction.

MATERIALS AND METHODS

Animals

Seven-week-old male Sprague Dawley specific-pathogen free rats ($n = 24$) weighing 200-250 g were divided into a control group and an experimental group. Sham ($n = 6$) is a control group for observing spontaneous healing after induction of oral ulcers, and the experimental group consists of 3 groups: Laser ($n = 6$), Liquiband ($n = 6$), Laser +

Liquiband ($n = 6$). A total of 24 animals were treated after oral ulcer induction. The study was performed according to the guidelines of the Institutional Animal Care and Use Committee at Dankook University Medical School (Approval No. DKU-20-026).

Laser & liquiband

The laser used for the treatment was WELS 01 of Wels Meditech (Cheonan, Korea) at 808-nm wavelength, and the Liquiband product was Liquiband Exceed (Hanmi Healthcare, Seoul, Korea).

Weight measurement

Body weights of all experimental animals were measured at 0, 2, 4, 6, and 8 days after induction of oral ulcer. The change in body weight was measured to confirm the degree of pain relief and stability.

Oral ulcer induction protocol

Each rat was anesthetized by intraperitoneal injection 300 mg/kg of 0.25% Avertin and Rompun diluted solution, and then 15 μ l of 60% acetic acid was injected into the right oral mucosa by intradermal injection. After confirming the formation of ulcers, treatment was performed (Fig. 1A).

The method of laser irradiation and liquiband treatment

Sham was a group that observed spontaneous healing after induction of oral ulcer. The size of the ulcer site was measured on days 0, 2, 4, 6, and 8. The laser group used a retractor to open the mouth and irradiated the ulcer with a laser with the same power (50 mW) and energy (4 J/time) for 240 seconds on the 0, 2, 4, 6, and 8 days, and then measured the size of the ulcer (Fig. 1B). The Liquiband group was difficult to access due to the size of the product to rat ulcers, so 10 μ l of Liquiband liquid was directly treated on the 0, 2, 4, 6, and 8 days using a pipette, and then measured the size of the ulcer (Fig. 1C). In the Laser + Liquiband group, laser and Liquiband under the same conditions as the previous two groups were treated on days 0, 2, 4, 6, and 8, and then measured the size of the ulcer.

Confirmation of oral ulcer recovery effect

Microscopic observation

After inducing oral ulcer, the cross-sectional area of the ulcer was visually observed at 0, 2, 4, 6, and 8 days. The area of the ulcer was compared by measuring the long diameter and the short diameter of the ulcer in consideration of the shape not being healed uniformly. The

Fig. 1. Images showing (A) chemical induction of oral ulcers using acetic acid in a rat model, (B) laser device and laser treatment, and (C) Liqui-band treatment.

ulcer healing rate was calculated by measuring the area of the ulcer immediately after the induction of oral ulcer (U0) and the area measured during subsequent visual observation (Ui) with the following formula.

$$\text{Recovery of Ulcer Area (\%)} = (U0 - Ui) / U0 \times 100$$

Histological analysis

Oral ulcer tissues were collected on the 5th and 9th days after the treatment. After hematoxylin and eosin (H&E) staining and Masson's trichrome staining, histological observation was performed under an optical microscope.

Statistical analysis

Weight change and oral ulcer size in each experimental group were performed by a two-way ANOVA test (bonferroni post-test) using Prism 5.0 (GraphPad, USA) analysis software, and if the *p*-value was less than 0.05, it was considered a significant difference.

Tissue staining method

H&E staining

After fixing the oral ulcer tissue in 4% PFA solution, make a paraffin block and attach the sectioned tissue (4 µm) to the slide. The tissues were deparaffinized using 100% xylene solution and 100, 95, 90, 70% alcohol solutions. Then, hematoxylin solution was treated for 5 minutes, washed with tap water for 10 minutes, treated with 1% acid alcohol for 30 seconds, and eosin for 20 seconds to perform tissue staining. The stained tissue was dehydrated with 70, 90, 95, 100% alcohol solutions and xylene solution, and then embedding. The H&E staining tissue was observed with an optical microscope. H&E staining was performed to confirm the degree of re-epithelialization and infiltration of inflammatory cells through struc-

tural observation of the oral ulcer tissue.

Masson's trichrome staining

After fixing the oral ulcer tissue in 4% PFA solution, make a paraffin block and attach the sectioned tissue (4 µm) to the slide. The tissues were deparaffinized using 100% xylene solution and 100, 95, 90, 70% alcohol solutions. Then, Weigert iron hematoxylin solution was treated for 10 minutes, washed with 40-50°C warm water for 3 minutes, treated with Biebrich scarlet-Acid fuchsin solution for 5 minutes, Phosphotungstic-Phosphomolybdic acid solution for 10 minutes, Aniline blue solution for 3 minutes, washed with tap water for 5 minutes to perform tissue staining. This staining was performed using Trichrome Stain Kit Masson, Aniline Blue (BBC Biochemical, USA). The stained tissue was dehydrated with 70, 90, 95, 100% alcohol solutions and xylene solution, and then embedding. Masson's trichrome staining was performed to confirm the extent of healing by observing collagen in the ulcer tissue. The Masson's trichrome staining tissue was observed with an optical microscope.

RESULTS

In this study, we found that laser treatment and Liqui-band application improved oral ulcer healing in vivo especially when combined.

Recovery effect confirmed by weight change and size of ulcer

As a result of measuring the weight of all experimental animals during the observation and treatment period, there was no significant difference in weight change in the control group and the experimental group, and there was a tendency to increase overall (Fig. 2A). Through this, pain relief of oral ulcers can be inferred with no observable

Fig. 2. (A) Weight changes in experimental animals after treatment. (B) Recovery of ulcer area (%) after laser, Liquiband, or combined laser + Liquiband treatment of oral ulcer animal models taken at 2, 4, 6 and 8 days. Statistical significance was assessed by two-way ANOVA test with bonferroni post-test. * $p \leq 0.05$, ** $p \leq 0.01$, *** $p \leq 0.001$, **** $p \leq 0.0001$ at $n=6$.

Fig. 3. Photographs of oral ulcer area showing changes on the surfaces of the ulcers after treatment with laser, Liquiband, or combined laser + Liquiband taken at 2, 4, 6 and 8 days. Reduction in ulcer area with time and earlier disappearance of yellowish-white membrane on the surface of ulcers was observed for combined treatment group (scale bar: 2 cm).

adverse effects from each treatment (laser, Liquiband or combination) relative to control group. Initially (day 2), recovery of ulcer area was only significantly different for laser treatment and combined (laser with Liquiband application) relative to control group (Fig. 2B). This implies the potential effects of laser treatment in initiating wound

healing. However, this did not persist and Liquiband treatment groups showed significantly higher recovery of ulcer area from the second treatment onwards. This may be due to difference in the effects of either laser or Liquiband in the mode of oral ulcer healing. However, it is important to note that the combined treatment showed significantly

better recovery relative to control in all observation time-points in contrast with Liquiband, which was not significantly different at day 4.

Further observation showed variances in the progression of healing of the ulcer area surface. The surface of the wounds was observed through images of the ulcer area taken at different time points post treatment. It can be observed from the images that ulcer area has reduced at day 4, and swelling appear to subside as well. However, the disappearance of the yellowish white pseudomembrane was more prominent for the combined treatment (Fig. 3), as can be observed at day 6 and 8. Further observation through histological analysis was then performed to get a proper insight on the healing progression occurring in the treatment groups.

Inflammatory reaction

Inflammatory cells were observed in all tissue samples at day 5. This was attributed to the induced inflammation

due to chemical treatment of the oral mucosa. At day 10, persistence of inflammatory cells can be observed for all treatment groups relative to control. But the presence of granulation tissue with vascularization (Fig. 4), which is a characteristic of wound healing was more evident with Liquiband and combined treatment groups. This supports the observed enhanced wound healing for either Liquiband or combined treatment group in terms of ulcer recovery.

Collagen matrix synthesis and formation

Collagen was prominent for all treatment groups relative to control group based on tissue samples stained with Masson's trichrome (Fig. 5). However, at day 10 collagen was only persistent for laser treated and combined treatment groups. This is indicative that laser treatment effects result in stimulation of collagen synthesis which is also known to enhance wound healing.

Fig. 4. Histological analysis of inflammatory reaction in tissue samples extracted at 5 and 10 days through hematoxylin and eosin (H&E) staining (original magnification $\times 200$, scale bar: 100 μm). Inflammatory cells were observed in all tissue samples at day 5 with persistent inflammation at day 10 for treated samples. Granulation tissue and vascularization (black arrow) was observed for treated groups relative to control.

Fig. 5. Collagen synthesis and formation observed through Masson's trichrome staining of tissue samples extracted at 5 and 10 days (original magnification $\times 200$, scale bar: 100 μm). Collagen (blue) was prominent with treated groups relative to control, especially for laser treated and combined treatment groups.

DISCUSSION

Recovery of oral soft tissue occurs in a continuous and complex phase, and new cells and tissues are reformed through processes such as inflammation, proliferation, and differentiation.¹⁵ When ulcers heal, extracellular matrix such as collagen, fibronectin, proteoglycan, and matricellular protein react with cells. This leads to the formation of a new matrix in the tissue and the remodeling of cells.¹⁶ Therefore, the degree of healing can be confirmed by observing the distribution of inflammatory cells or collagen in the ulcer tissue.^{17,18}

Inflammatory diseases of the oral mucosa cause pain during everyday life, such as eating, swallowing, and talking.¹⁹ Oral ulcers from aphthous stomatitis are relatively small in diameter and heal without scarring within 7-14 days, but the pain is often not proportional to size.²⁰ Since this causes severe pain even with a small stimulus, treatment using a laser or treatment to form a protective film is required. In addition, low-power laser treatment has been applied to diabetic patients with few side effects and slow wound healing.^{6,21} As such, laser treatment has a clear effect on the treatment of ulcers, but the results may vary depending on the wavelength or output of the laser and the time of treatment. Therefore, it is necessary to conduct further research by finding the optimal conditions with high treatment effect for each laser. However, results from this study have shown that laser treatment effect is mainly attributed to collagen synthesis and Liquiband treatment seem to promote vascularization and granulation tissue formation. Thus, the combined treatment upon further optimization could significantly enhance oral ulcer healing synergistically.

CONCLUSION

In the treatment of ulcers, topical application of 808-nm Laser or Liquiband showed different effects, but both methods confirmed significant effects relative to untreated group. The combined treatment group with laser and Liquiband also showed marked oral ulcer healing but further optimization is required to get highly significant results and establish potential synergistic effect of the combined treatment as compared with individual effects to enhance oral ulcer healing.

CONFLICT OF INTEREST

Seung Hoon Woo is the Editor-in-Chief of the journal but was not involved in the review process of this manuscript.

Celine Abueva and Phil-Sang Chung are editorial board members of the journal but were not involved in the review process of this manuscript. Otherwise, there is no conflict of interest to declare.

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