Photomedicine and Laser Surgery
Volume XX, Number XX, 2017
© Mary Ann Liebert, Inc.
Pp. 1–5
DOI: 10.1089/pho.2017.4325

Original Research

Different Protocols of Photobiomodulation Therapy of Hyposalivation

Božana Lončar Brzak, DMD, PhD,[1] Livia Cigić, DMD, PhD,[2] Marinka Baričević, DMD, PhD,[3] Ivan Sabol, PhD,[4] Marinka Mravak-Stipetić, DMD, PhD,[1] and Dubravko Risović, PhD[5]

Abstract

Aim: Photobiomodulation (PBM) therapy has proved to be effective for a wide range of oral pathologies including oral dryness, but the literature still lacks reports of clinical trials and protocols. The purpose of our study was to evaluate the effects of different wavelengths of PBM on salivation in patients suffering from hyposalivation aiming at determination of optimal treatment protocol. ***Materials and methods:*** This study included 30 patients whose major salivary glands were treated with low-intensity diode laser BTL2000 (Medical Technologies, s.r.o., Czech Republic) during 10 consecutive days. Patients were randomly assigned into two groups, each of 15 patients, and treated with PBM of 830 nm and PBM of 685 nm, respectively. The whole unstimulated and stimulated saliva quantities were measured each day during 10 days, before and after laser treatment, and at 10th day after treatment was ended. ***Results:*** Results have shown that the laser treatment significantly improves salivation ($p < 0.0001$) in both groups after 10 days treatment. The salivation also remains improved 10 days after the end of treatment. The patients treated with PBM of 830 nm have had continuously higher values of quantity of saliva. ***Conclusions:*** Our results have shown that both laser wavelengths were effective in increasing salivary flow rate, and the improvement in salivation was statistically significant. The effect of treatment could be observed 10 days after the completion of treatment, thus providing evidence not only of stimulative effect but also indicating regenerative potential of PBM therapy.

Keywords: photobiomodulation, protocol, hyposalivation

Introduction

Xerostomia is an often complaint mainly in elderly population, resulting in difficulties in speaking, food swallowing and tasting, denture wearing, burning sensations in the mouth, and increased susceptibility of oral mucosa to diseases.[1] Although the lack of saliva seriously impairs the quality of life, it is often neglected. Etiology of xerostomia is different, varying from salivary gland disease, systemic diseases, radiation therapy or, the most frequently, drug-induced xerostomia.[2–5] Increasing salivary flow (in case acinar cells are preserved) represents the best therapy, but gustatory stimulation is short acting and must be often repeated.[6,7] Other therapeutic models include use of systemic sialogogues, electrical stimulation, acupuncture,[3] and use of saliva substitutes.[5] Each of these methods has some limitations, as described in our previous work.[8] Many different artificial saliva products on the market are of limited value in the treatment of xerostomia. Although convenient and simple, some patients find gustatory stimulation debilitating and tiresome. Systemic cholinergic agents such as pilocarpine and cevimeline increase salivary flow rate, but have side effects and certain contraindications.[5]

Low-level laser treatment, more recently termed photobiomodulation (PBM), has proved to be effective for a wide range of oral pathologies including oral dryness, but the literature still lacks reports of clinical trials and protocols. PBM therapy induces changes in the cellular redox state and pH homeostasis,[9] increases ATP production in the cell,[10,11] and converts laser light energy input through biochemical and photophysical processes into energy useful to the cell.[12]

[1]Department of Oral Medicine, School of Dental Medicine, University of Zagreb, Zagreb, Croatia.
[2]Department of Oral Medicine and Periodontology, School of Medicine, University of Split, Split, Croatia.
[3]Health Care Centre Zagreb West, Zagreb, Croatia.
[4]Department of Molecular Medicine, Ruđer Bošković Institute, Zagreb, Croatia.
[5]Molecular Physics Laboratory, Ruđer Bošković Institute, Centre of Excellence for Advanced Materials and Sensing Devices, Zagreb, Croatia.

In our previous study,[8] we have reported the efficacy of PBM for the treatment of xerostomia, providing good therapeutic results. Since then, we have introduced a new laser device and performed research with two different wavelengths in patients with hyposalivation. The aim was to evaluate the effects of these new laser wavelengths, different from those used in previous research, on salivation of patients suffering from hyposalivation. This is with the purpose to possibly find the optimum laser wavelength for the treatment and to determine a better protocol for the patients suffering from hyposalivation.

Materials and Methods

The study included 30 consent female patients (age range 52–85 years, mean age 69.6, median 72) whose major salivary glands were treated with low-intensity diode laser (BTL-2000 Medical Technologies, Prague, BTL Co., Czech Rep.). The nominal output of the probes were 30 mW at 685 nm and 100 mW at 830 nm and set at a pulse repetition rate of 5.2 Hz to deliver 30 mW at 685 nm and 35 mW at 830 nm. The power measurements were provided by the manufacturer. The treatment was performed for 10 consecutive days with a laser probe. During application, protective eyeglasses were worn both by the operator and the patient. The patients were divided into two groups: the first group comprised 15 female patients (age range 52–77 years, mean age 69.53, median 72) who were treated with 830 nm laser wavelength. The second group comprised 15 female patients (age range 55–85 years, mean age 67.67, median 72) who were treated with 685 nm laser wavelength. To establish the diagnosis of hyposalivation, the amount of total unstimulated saliva was measured at the initial examination of the patients. After that the salivation was stimulated with citric acid. PBM therapy was not started at the initial examination of the patient to avoid any possible error in the measurement of saliva quantity after different types of stimulation. The PBM therapy began on the next day.

The total unstimulated and stimulated saliva quantities were measured before and after each laser treatment and 10 days after the last (10th) treatment to determine the durability of results. Patients were asked to expectorate all saliva into graduated test tubes for a 5-min period. The amount of saliva was determined by the scale on the graduated tubes.

The inclusion criteria for all patients were medical histories free of radiotherapy and Sjögren's syndrome. Sjögren's syndrome was excluded by the applied diagnostic criteria.[13] In all patients, clinical examination and sialometry were done and anamnestic data (drug history) were taken. Our research was approved by the Ethical Committee of our faculty and registered at U.S. National Institutes of Health (trial identifier: NCT03049943). All patients signed informed consent to participate in the study.

The laser beam was applied bilaterally to each salivary gland area, extraorally to the parotid and submandibular glands and intraorally to the sublingual gland (each patient received a total of six exposures, each of different duration depending of the gland and laser wavelength that was predetermined by the laser settings). The treatment lasted for 10 consecutive days. The distance between the probe and the irradiated area was kept constant at 0.5 cm throughout the treatment period. However, the spot size was difficult to calculate because of very slight differences in distance from the irradiated area (due to manual probe shifts) and beam divergence. The laser probe was not held stationary, but moved around in mesh-shaped movements. This technique is recommended for manual scanning to ensure that a reasonably uniform dosage is delivered to all areas of the treated surface.[14] The relevant technical data for the PBM therapy are given in Table 1.

Statistical methods, including correlation analysis, *t*-test, and repeated measures ANOVA (with Bonferroni's multiple comparison *post hoc* test), were used for comparison and interpretation of the difference in salivary flow rates between the two groups.

Results

The average salivary flow rates obtained from both laser wavelength groups before and after PBM throughout the 10-day treatment period and 10 days after the last (10th) treatment are shown in Fig. 1. If we compare the results by the laser wavelength, we can see that the patients in the 830 nm laser wavelength group have higher values of saliva

Table 1. Relevant Technical Data for Laser Therapy

Salivary gland	*Laser specifications*	*685 nm*	*830 nm*
Parotid gland	Dose (J/cm^2)	1.80	1.80
	Power (mW)	30	35
	Area (cm^2)	4.00	4.00
	Applied energy (J)	9	8.995
	Power density (W/cm^2)	0.0075	0.00875
	Distance (cm)	0.5	0.5
	Time (min:sec)	5:00	4:17
	Max. power (mW)	30	35
	Frequency (Hz)	5.20	5.20
	Number of treatments	10	10
	Cumulative dose given (J/cm^2)	18.0	18.0
Submandibular gland	Dose (J/cm^2)	1.80	1.80
	Power (mW)	30	35
	Area (cm^2)	1.60	1.60
	Applied energy (J)	3.6	3.605
	Power density (W/cm^2)	0.01875	0.021875
	Distance (cm)	0.5	0.5
	Time (min:sec)	2:00	1:43
	Max. power (mW)	30	35
	Frequency (Hz)	5.20	5.20
	Number of treatments	10	10
	Cumulative dose given (J/cm^2)	18.0	18.0
Sublingual gland	Dose (J/cm^2)	1.80	1.80
	Power (mW)	30	35
	Area (cm^2)	0.80	0.80
	Applied energy (J)	1.8	1.785
	Power density (W/cm^2)	0.0375	0.04375
	Distance (cm)	0.5	0.5
	Time (min:sec)	1:00	0:51
	Max. power (mW)	30	35
	Frequency (Hz)	5.20	5.20
	Number of treatments	10	10
	Cumulative dose given (J/cm^2)	18.0	18.0

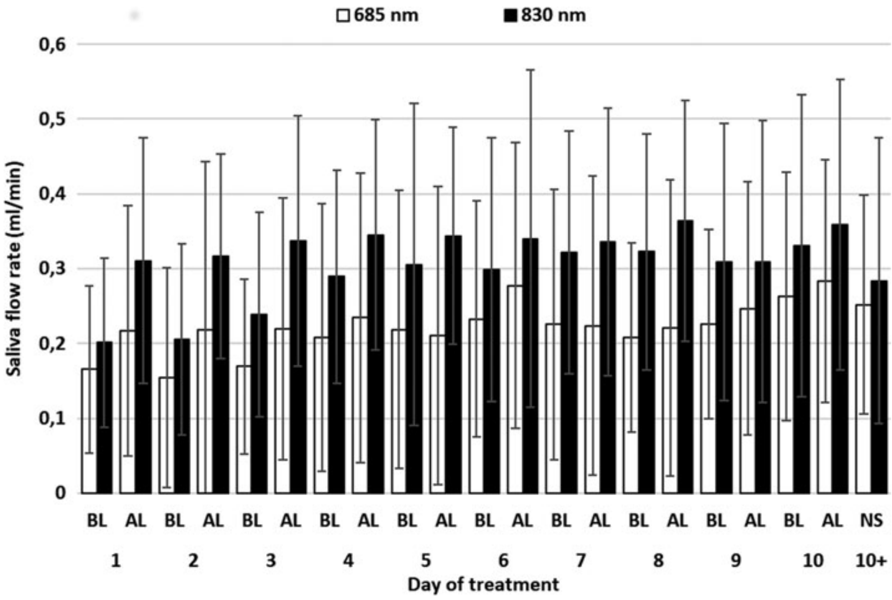

FIG. 1. Average measured mean saliva flow rate in groups treated with different lasers: BL, before laser treatment, AL, after laser treatment and 10 days after completion of treatment series (NS).

stimulation throughout the study than the patients in the 685 nm laser wavelength group.

The comparison of average measured salivary flow rate before laser treatment on the first day with that on the last day of treatment series shows significantly higher rate for both laser groups of patients and for all patients [*t-test for paired examples*, $p = 0.0044$ (685 nm), $p = 0.0019$ (830 nm), $p < 0.0001$ (all patients)] (Fig. 2.). Also the comparison of average measured salivary flow rate before laser treatment on the 1st day with that measured 10 days after the end of the treatment shows that salivation was significantly increased for all patients (*t*-test for paired examples, $p = 0.0009$) and each group of patients [$p = 0.0121$ (685 nm), $p = 0.0347$ (830 nm)] (Fig. 3.). The results were also statistically significant by repeated measurements ANOVA testing (all patients $p < 0.0001$; 685 nm laser $p = 0.001$; 830 nm laser $p < 0.0001$). However, Bonferroni's multiple comparison *post hoc* test only confirmed the significance of salivary flow rate differences between the first day and last day of treatment for the 830 nm laser ($p < 0.01$). The difference between 1st day and 10th day after treatment did not reach statistical significance together with same comparisons for 685 nm laser.

Discussion

Our results show that treatment with both laser wavelengths, 830 and 685 nm, was effective in improving the salivary flow rate. After 10 days of treatment in each treated group of patients, the average salivary flow rate was significantly higher. The treatment with wavelength of 830 nm was more effective, resulting in a higher average increase of

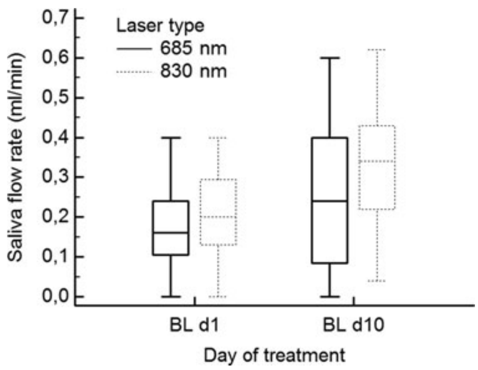

FIG. 2. The difference in measured whole unstimulated saliva flow rate (BL, before the laser treatment) on the first and on the last day of treatment. BL d1 = unstimulated saliva flow rate on the first day of treatment, before laser treatment; BL d10 = unstimulated saliva flow rate before laser treatment on the 10th day of treatment [*t*-test for paired examples, $p = 0.0044$ (685 nm), $p = 0.0019$ (830 nm), $p < 0.0001$ (all patients)].

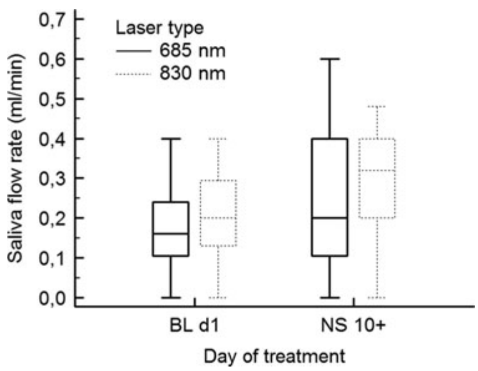

FIG. 3. The difference in measured whole unstimulated saliva flow rate before laser treatment on the first day of treatment (BL d1) and 10 days after the end of treatment. BL d1 = salivary flow rate before laser therapy on the first day; NS 10+ = salivary flow rate 10 days after completion of laser therapy [*t*-test for paired examples, $p = 0.0121$ (685 nm), $p = 0.0347$ (830 nm) and all patients ($p = 0.0009$)].

salivation. The observed difference in efficiency of 830 and 685 nm (at the same applied energy level) could be attributed to different spectral absorption properties of the oral soft tissue's main chromophores, in particular water and oxyhemoglobin. Namely, the absorption coefficient of water (comprising about 75% of the tissue) is approximately five times higher at 830 nm than at 685 nm, whereas for oxyhemoglobin the difference in absorption coefficients is about 25%.[15,16]

By comparing the differences in saliva stimulation in each group for 10 consecutive days of treatment, we can see that the response of the gland to the same amount of applied energy was not constant. Unlike our previous study,[8] we did not observe linear increase in salivation flow during the treatment. Rather, after initial significant increase in first few days of treatment, it remained, with slight oscillations, at a relatively constant value. The salivation in both groups on the last day of treatment and 10 days after the end of the treatment was improved, in comparison with the unstimulated whole saliva on the first day of treatment before beginning of PBM therapy, and this difference is statistically significant. The salivation remained improved for prolonged time as documented with measurements accomplished 10 days after the end of treatment. This improvement in the salivation rate, although somewhat lower than the increase observed during the treatment, is important because it indicates that this type of therapy does not have only short-term effect, but has also the regenerative effect on salivary glands as their function remains restored for a certain time after the completion of therapy. In recent years, the regenerative effect of PBM was described in the literature for treatment of muscle repair,[17,18] wound healing,[19,20] mesenchymal stromal cells therapies,[21] and xerostomia.[8] Our results also confirm the biostimulative and regenerative effect of PBM, because of restoring salivary glands function, which remained improved at least 10 days after the termination of the PBM therapy. In this context, it would be intriguing to see whether an occasional PBM therapy would help to maintain the achieved amount of salivation on constant level, which would be a great advantage of this type of hyposalivation treatment.

Regarding the effects of medication on salivation in the literature, one can find many studies reporting decrease of salivary flow rate because of use of various drugs.[2,22–24] Since all of our patients were taking medication for their other health conditions, we could assume that the xerogenic effect of the used drugs, with prolonged time after the completion of therapy, inhibits salivation, causing hyposalivation. Despite this possible negative influence of used drugs, our investigation proved the effectiveness of the 830 and 685 nm PBM therapy in stimulation of salivation.

Since the world population of elderly people is increasing and polytherapy (taking multiple medications) is becoming more and more frequent in the general population, it is important to find efficient therapy for hyposalivation, which does not have side effects and gives the best results. In this context, it is important to emphasize that none of the patients included in this study reported any side effects from this type of therapy. The literature offers many positive results of PBM for treatment of decreased salivary flow rate,[8,25–29] but with different laser parameters and treatment protocol. Searching the literature, we did not find published research on patients with hyposalivation similar to ours, so we could not compare our results with those from other studies. In comparison with our previous study wherein we have applied 904 nm wavelength on salivary glands,[8] along with these results, we can confirm better therapeutic response of wavelengths in the infrared part of spectrum.

Conclusions

We have investigated the effects of PBM with wavelengths 830 and 685 nm on salivation in patients suffering from hyposalivation.

Our results have shown that both laser wavelengths with selected exposure times were effective in increasing salivary flow rate, and that the improvement in salivation was significant. Although the difference between the two laser groups was not statistically highly significant, the 830 nm laser wavelength proved to be more effective in stimulating salivation. This difference could be attributed to the different spectral absorption properties of the oral soft tissue's main chromophores. The positive effect of the PBM therapy could be observed 10 days after completion of treatment, with better maintained effect of 830 nm wavelength. Thus indicating a regenerative potential of this type of therapy that should be further investigated and compared with other therapeutic options for hyposalivation.

Author Disclosure Statement

No competing financial interests exist.

References

1. Pedersen A, Bardow A, Jensen S, Nauntofte B. Saliva and gastrointestinal functions of taste, mastication, swallowing and digestion. Oral Dis 2002;8:117–129.
2. Llena-Puy C. The role of saliva in maintaining oral health and as an aid to diagnosis. Med Oral Patol Cir Bucal 2006;11:E449–E445.
3. Grisius MG, Fox P. Salivary gland diseases. In: Burket's Oral Medicine, Diagnosis and Treatment. Greenberg MS, Glick M (eds.). Hamilton, ON, Canada: BC Decker, Inc., 2003; pp. 235–270.
4. Turner MD, Ship JA. Dry mouth and its effects on the oral health of elderly people. J Am Dent Assoc 2007;138:5–20.
5. Cassolato SF, Turnbull RS. Xerostomia: clinical aspects and treatment. Gerodontology 2003;20:64–77.
6. Garg AK, Kirsh ER. Xerostomia: recognition and management of hypofunction of the salivary glands. Compend Contin Educ 1995;16:574–583.
7. Blom M, Dawidson I, Angmar-Månsson B. The effect of acupuncture on salivary flow rates in patients with xerostomia. Oral Surg Oral Med Oral Pathol 1992;73:293–298.
8. Lončar B, Mravak-Stipetić M, Baričević M, Risović D. The effect of low-level laser therapy (LLLT) on salivary glands in patients with xerostomia. Photomed Laser Surg 2011; 29:171–175.
9. Karu T. Lecture 1- Introductory remarks. Where are we and whither do we go? In: Ten Lectures on Basic Science of Laser Phototherapy. Nobel A (ed.) Grängesberg, Sweden: Sweden Prima Books AB, 2007; p. 4.
10. Yu W, Naim JO, McGowan M, Ippolito K, Lanzafame RJ. Photomodulation of oxidative metabolism and electron chain enzymes in rat liver mitochondria. Photochem Photobiol 1997;66:866–871.
11. Passarella S. He-Ne laser irradiation of isolated mitochondria. J Photochem Photobiol B 1989;3:642–643.

12. Mizutani K, Musya Y, Wakae K, et al. A clinical study on serum prostaglandin E2 with low-level laser therapy. Photomed Laser Surg 2004;22:537–539.
13. Vitali C, Bombardieri S, Jonsson R, et al. Classification criteria for Sjögren's syndrome: a revised version of the European criteria proposed by the American–European Consensus Group. Ann Rheum Dis 2002;61:554–558.
14. Baxter D. Principles and practice of laser treatment. In: Therapeutic Lasers, Theory and Practice. Hunter S (ed.). New York: Churchill Livingstone, 1997; pp. 187–220.
15. Hale G, Querry M. Optical constants of water in the 200-nm to 200-microm wavelength region. Appl Opt 1973;12:555–563.
16. Jacques S. Optical properties of biological tissues: a review. Phys Med Biol 2013;58:R37–R61.
17. da Silva Neto Trajano, LA Stumbo A, da Silva C, Mencalha A, Fonseca A. Low-level infrared laser modulates muscle repair and chromosome stabilization genes in myoblasts. Lasers Med Sci 2016;31:1161–1167.
18. Mesquita-Ferrari R, Alves A, de Oliveira Cardoso V, et al. Low-level laser irradiation modulates cell viability and creatine kinase activity in C2C12 muscle cells during the differentiation process. Lasers Med Sci 2015;30:2209–2213.
19. Mester E, Spiry T, Szende B, Tota J. Effect of laser rays on wound healing. Am J Surg 1971;122:532–535.
20. da Silva J, da Silva M, Almeida A, Lombardi Junior I, Matos A. Laser therapy in the tissue repair process: a literature review. Photomed Laser Surg 2010;28:17–21.
21. Kushibiki T, Hirasawa T, Okawa S, Ishihara M. Low reactive level laser therapy for mesenchymal stromal cells therapies. Stem Cells Int 2015;2015:974864.
22. Delli K, Spijkervet F, Kroese F, Bootsma H, Vissink A. Xerostomia. Monogr Oral Sci 2014;24:109–125.
23. Gómez-Moreno G, Aguilar-Salvatierra A, Guardia J, et al. The efficacy of a topical sialogogue spray containing 1% malic acid in patients with antidepressant-induced dry mouth: a double-blind, randomized clinical trial. Depress Anxiety 2013;30:137–142.
24. Mansourian A, Manouchehri A, Shirazian S, Moslemi E, Haghpanah G. Comparison of oral lesion prevalence between renal transplant patients and dialysis patients. J Dent 2013;10:487–493.
25. Carroll J, Milward M, Cooper P, Hadis M, Palin W. Developments in low level light therapy (LLLT) for dentistry. Dent Mater 2014;30:465–475.
26. Simões A, Platero M, Campos L, Aranha A, Eduardo C, Nicolau J. Laser as a therapy for dry mouth symptoms in a patient with Sjogren's syndrome: a case report. Spec Care Dent 2009;29:134–137.
27. Campos L, Simões A, Sá P, Eduardo CP. Improvement in quality of life of an oncological patient by laser phototherapy. Photomed Laser Surg 2009;27:371–374.
28. Gonnelli F, Palma L, Giordani A, et al. Low-Level Laser for Mitigation of Low Salivary Flow Rate in Head and Neck Cancer PatientsUndergoing Radiochemotherapy: a Prospective Longitudinal Study. Photomed Laser Surg 2016;34:326–330.
29. Simões A, de Campos L, de Souza D, de Matos J, Freitas P, Nicolau J. Laser phototherapy as topical prophylaxis against radiation-induced xerostomia. Photomed Laser Surg 2010;28:357–363.

Address correspondence to:
Marinka Mravak-Stipetić
Department of Oral Medicine
School of Dental Medicine
University of Zagreb
Gundulićeva 5
Zagreb 10000
Croatia

E-mail: mravak@sfzg.hr

Received: April 21, 2017.
Accepted after revision: July 21, 2017.
Published online: October 9, 2017.