Lasers in Medical Science (2018) 33:1813–1819
https://doi.org/10.1007/s10103-018-2549-y

ORIGINAL ARTICLE

Treatment of mucositis with combined 660- and 808-nm-wavelength low-level laser therapy reduced mucositis grade, pain, and use of analgesics: a parallel, single-blind, two-arm controlled study

Raquel Gusmão Soares[1] · Lucyana Conceição Farias[1] · Agna Soares da Silva Menezes[1] · Carla Silvana de Oliveira e Silva[2] · Angeliny Tamiarana Lima Tabosa[1] · Pablo Vinicyus Ferreira Chagas[1] · Laís Santiago[3] · Sérgio Henrique Sousa Santos[4] · Alfredo Maurício Batista de Paula[1] · André Luiz Sena Guimarães[1,3,5]

Received: 4 January 2018 / Accepted: 21 May 2018 / Published online: 15 June 2018

Abstract

Oral squamous cell carcinoma (OSCC) is the most frequent oral malignant neoplasia. As consequence of OSCC treatment, oral mucositis (OM) is one of the most common adverse effects of OSCC treatment. Currently, there is no consensus for OM treatment. The purpose of the current study was to test the combination of red and infrared low-level laser therapy (LLLT) for OM treatment. Primary culture of human fibroblast was performed to identify LLLT dose. After laboratory tests, a two-arm parallel, single-blind, controlled study was conducted. The two arms were group 1, both 660- and 808-nm wavelengths (300 J/cm^2, 9 J of total energy, 100 mW, spot size 3 mm^2), and group 2, only 660-nm wavelength (300 J/cm^2, 9 J of total energy, 100 mW, spot size 3 mm^2). Both treatments were performed twice a week. Group 1 presented a reduction of mucositis grade in comparison to group 2. Group 1 also presented reduction of analgesics prescription. But no significant differences between groups 1 and 2 were observed according to the pain scale. In conclusion, the current study demonstrated that a combination of red and infrared at a higher dose (300 J/cm^2) reduced both oral mucositis grade and analgesics prescription. The effects of the combination of RT and LLLT are unclear and need more studies.

Keywords LASER · Light therapy · LED · Phototherapy · Radiation · Cancer · Pain · Mouth

Introduction

Oral squamous cell carcinoma (OSCC) is the most common type of oral malignant neoplasia [1, 2]. OSCC treatment is associated with dysfunction and distortions of head and neck structures [3, 4]. Moreover, OSCC treatment also causes oral mucositis (OM) [5]. OM is graded into four categories according to the World Health Organization (WHO) [6]. Head and neck radiation therapy (RT) is responsible for worse mucositis (grades 3 and 4) in 50% of patients [6]. The main consequences of OM are pain, eating and swallowing disorders, and gastrointestinal distress [5, 6].

There are a vast number of suggested treatments for OM including oral cryotherapy [7], the use of recombinant human keratinocyte growth factor-1 [8], nonsteroidal anti-inflammatory drugs (NSAID) [9], opioids [10], and low-level laser therapy (LLLT) [11]. Despite different treatment options, OM is still a frequent complication decurrent of head and neck cancer treatment [12]. Differences in parameters, such as wavelength and dose, are responsible for a plethora of results regarding LLLT in OM treatment in the literature [13]. LLLT has different effects in OM treatment according to the wavelength [13]. According to wavelength, LLLT acts

✉ André Luiz Sena Guimarães
andreluizguimaraes@gmail.com

[1] Department of Dentistry, Universidade Estadual de Montes Claros (Unimontes), Montes Claros, Minas Gerais, Brazil

[2] Department of Nursy, Universidade Estadual de Montes Claros (Unimontes), Montes Claros, Minas Gerais, Brazil

[3] Dilson Godinho Hospital, Montes Claros, Minas Gerais, Brazil

[4] Institute of Agricultural Sciences, Universidade Federal de Minas Gerais (UFMG), Montes Claros, Minas Gerais, Brazil

[5] Universidade Estadual de Montes Claros, Laboratório de Pesquisa em Saúde, Hospital Universitário Clemente Faria, 562 Av. Cula Mangabeira Santo Expedito, Montes Claros, Minas Gerais 39401-001, Brazil

differently in the cells [14–18]. For example, red and infrared light energy induce photon absorption, but both wavebands have different primary targets and photoreactions in target cells [14–19]. The red light is mainly absorbed by cytochrome c oxidase while infrared is incorporated in the plasmatic membrane by a photophysical reaction in the cell membrane [13–19]. It was demonstrated that LLLT at three times superior dose reduced pain and prevented OM [11]. Interesting enough, LLLT with a dose of 2.5 J/cm^2 of 660 nm did not prevent OM, but LLLT reduced RT interruptions [20]. The benefit of combining different wavelengths was demonstrated before [21, 22]. However, most of the guidelines and systematic reviews only focus on LLLT with red wavelength [10, 23, 24]. In recent meta-analyses, no data of 808-nm LLLT were observed [24]. Also, LLLT could also reduce inflammatory mediators such as interleukin 1 and bradykinin which are associated with pain [25]. The absence of infrared studies in guidelines might be probably because the first studies with 808-nm wavelength used the unfocused high-level source to generate infrared LLLT [21]. The current study aims to test the protocol combining red and infrared LLLT to reduce pain in patients with oral mucositis.

Patients and methods

Dose establishment

For the dose establishment, primary culture assay was performed in triplicate as described before [26]. Briefly, primary fibroblast cells were isolated and cultured in Dulbecco's modified Eagle's medium (DMEM/ F12, GIBCO, Billings, MT, USA). The medium was supplemented with 10% fetal bovine serum (FBS, GIBCO, Billings, MT, USA), 400 ng/mL hydrocortisone, and 1% antibiotic solution (Invitrogen, Carlsbad, CA, USA). The cells were maintained at 37 °C in a humidified atmosphere of 5% CO_2; the growth area was 3.85 cm^2. The primary fibroblasts were stimulated with a laser equipment (GaA1As and InGaAlP dual diode LASER, LASER duo—MMOptics®, MMOptics Ltda., São Carlos, São Paulo, Brazil, RRID: SCR_015955) to compare the two conditions 300 J/cm^2 (90 s, 9 J of total energy, 100 mW, spot size 3 mm^2) and 3 J/cm^2 (9 s, 0.9 J of total energy, 100 mW, spot size 3 mm^2). Seven groups were used for comparison: group A (not submitted to laser irradiation), group B (cell irradiation with a dose of 3 J/cm^2 in red wavelength 660 nm), group C (cell irradiation with a dose of 300 J/cm^2 in red wavelength 660 nm), group D (cell irradiation with a dose of 3 J/cm^2 in infrared wavelength 808 nm), group E (cell irradiation with a dose of 300 J/cm^2 in infrared wavelength 808 nm), group F (cell irradiation with a dose of 3 J/cm^2 in a combined wavelength red and infrared), and group G (cell irradiation with a dose of 300 J/cm^2 in a combined wavelength red and infrared). The irradiation sessions were in continuous mode with a power of 100 mW and were carried out in partial darkness, without influence from other light sources. The irradiation was performed stationary in one session and direct contact with the plate. After 48 h of irradiation, the cells were then rinsed with sterile PBS followed by Masson's trichrome staining. Images were obtained in microscope FSX100 (Olympus, Center Valley, PA, USA) and quantified in the ImageJ software [27].

Study design

The current study is two-arm parallel, single-blind, and controlled. The number of patients was calculated according to the use of analgesics to OM. The considered values for sample calculation were alpha 0.05, beta 0.1, and study power 0.9 [28]. A total of 42 patients, between October 2015 and December 2016, met the criteria for participation in the study. All patients signed the informed consent. The data were collected in Dilson Godinho Hospital, Montes Claros, Brazil. Ethical approval for this study was obtained from the relevant Institutional Review Board (48215415.0.0000.5146). The study was also registered in the National Clinical Trials (UTN: U1111-1177-9023/67hjmn). The patients were blinded for intervention. The consecutive sampling randomization was adopted. The study recruiter was not a care provider. Also, the study recruiter did not know cancer staging or the proposed cancer treatment. Patients 1 to 20 were included in group 1 (red and infrared laser) and the numbers 21 to 42 composed group 2 (only red laser). The allocation concealment was performed by the study recruiter blinding both patients and all care providers. Group 1 consisted of 20 patients (16 males and 4 females), age range between 35 and 82 years (mean 58.5, SD 12.44). The distribution according to anatomical site presented 17 lesions located in the oral cavity and 3 in the oropharynx. According to the proposed treatment, all patients were submitted to RT, and 16 patients were subjected to chemotherapy. Group 2 was composed of 22 patients (20 males and 2 females), age range between 42 and 86 years (mean 61.2, SD 10.96). About tumor location, 18 were located in the oral cavity, 1 in the oropharynx, and 1 in the hypopharynx. According to the proposed treatment, all patients were submitted to RT, and 13 patients were submitted to chemotherapy. As inclusion criteria were patients with confirmed diagnosis of Squamous Cell Carcinoma in the oral cavity, oropharynx or Hypopharynx treated with RT exclusively with RT or associated with chemotherapy. Additionally, both 2D or 3D RT were accepted for the current study. The exclusion criteria were patients who have previously been submitted to RT in the head and neck, patients presenting Oral Mucositis during the enrollment, patients in using drugs that could interfere in OM, Cancer lesions associated with UV light, or patients who did not agree to participate in the study. The exclusion criteria were patients who have previously been submitted to RT in the head and neck, patients presenting Oral Mucositis during the enrollment, patients in using drugs that could interfere in OM, Cancer lesions associated with UV light, or patients who did not agree to participate in the study.

Oral mucositis grading and pain evaluation

Patients were evaluated twice a week, and mucositis was graded according to WHO [5, 6].

The oral pain was evaluated subjectively according to a visual analog scale (VAS), in which "0" is the absence of pain and "10" is the maximum pain. The patients were instructed to attribute a score to their degree of pain in oral mucositis as performed before [11]. Additionally, research in medical charts was also conducted to check if patients were in use of the drug for pain relief. Neither patients nor medicals knew about the groups.

Mucositis treatment

All groups were treated with the same instrument (GaA1As and InGaAlP dual diode LASER, LASERduo—MMOptics®, MMOptics Ltda., São Carlos, São Paulo, Brazil, RRID: SCR_015955) and the same professional. The treatment of mucositis per point was to group 1 both 660- and 808-nm wavelengths, 9 J of total energy, 100 mW, and spot size 3 mm^2; the total dose delivered to oral mucosa was 300 J/cm^2 twice a week. Mucositis lesions of group 2 were treated only with 660-nm wavelength, 9 J of total energy, 100 mW, and spot size 3 mm^2; the total dose delivered to oral mucosa was 300 J/cm^2 twice a week. Both groups were treated on Tuesdays and Thursdays. All patients have received instructions for oral care during radiation therapy. LLLT was applied stationary in direct contact with the lesions; it was used one point for every 4 cm^2 of the lesion. Extra-orally, LLLT was performed when complaints were located in inaccessible anatomic sites. The laser device power was evaluated once a week by a laser power meter (Aferidor Laser Check—MMOptics®, MMOptics Ltda., São Carlos, São Paulo, Brazil).

Statistical analysis

Kolmogorov–Smirnov and the Shapiro–Wilk tests were carried out to evaluate data distribution. After the definition of the data distribution, an appropriate test was conducted. These analyses revealed that the data were non-parametrically distributed; therefore, the Mann–Whitney and Kruskal–Wallis tests were performed. Chi-square and Fisher's exact frequency tests were applied for the statistical analysis of differences between groups 1 and 2 results. All statistical analyses were performed with PASW® v 18.0 for Windows®. Statistical significance was accepted at $p < 0.05$.

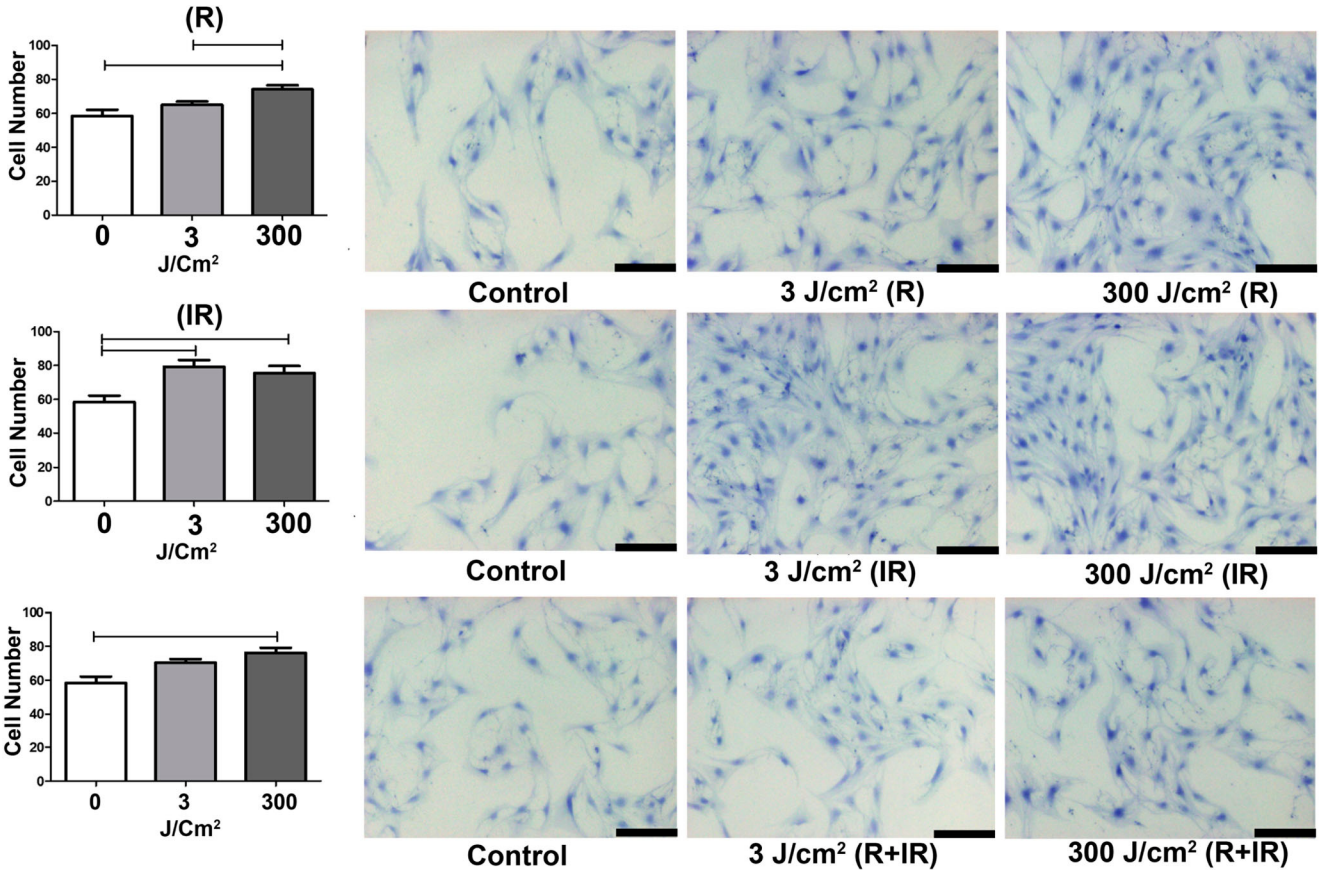

Fig. 1 Comparison of LLLT in fibroblasts. Lines represent significant p values. Scales represent 70 μm. (R) Red 660-nm and (IR) 808-nm wavelengths. The dose 300 J/cm^2 increased the number of fibroblast in an isolated or in a combination of wavelengths

Results

The comparison between doses of 3 and 300 J/cm^2 was performed in primary human fibroblast culture. The dose 300 J/cm^2 (90 s, 9 J of total energy, 100 mW, spot size 3 mm^2) increased the number of fibroblasts in an isolated or in a combination of wavelengths (red + infrared). While 3 J/cm^2 (9 s, 0.9 J of total energy, 100 mW, spot size 3 mm^2) presented a significant increase in number only in 808-nm wavelength (Fig. 1).

The trial flowchart is presented in Fig. 2. No differences between groups were observed according to sex, anatomical site, TNM staging, type of treatment, and RT dose (Table 1). The average of irradiation points per sessions was also similar between groups 1 and 2 (2.46 and 2.86 respectively data not shown). The evaluation of oral mucositis was performed twice a week, and the worse grade during the whole treatment was recorded to score oral mucositis. There were 6 (14.28%) patients who did not present clinical mucositis, 3 patients for each group (Table 2). Group 1 presented a reduction of mucositis grade in comparison to group 2 (Table 2). Grade 2 mucositis was the most common grade, committing 26 patients (61.90%) in both groups. It is important to highlight that in group 1, only 8 (40%) patients, while in group 2, 18 (81.81%) patients presented grade 2 mucositis. On the other hand, the combination of red and infrared LLLT increased the number of patients that developed grade 1 mucositis (Table 2).

No significant differences between groups 1 and 2 were observed according to pain scale (Table 3). However, the combination of red and infrared LLLT reduced the prescription of the analgesics drugs (Table 3). Also, no harms were observed in any group.

Discussion

OM is one of the most critical complications to the OSCC treatment [5, 6]. OM is associated with significant symptoms such as dysgeusia, dysphagia, and pain [29]. Moreover, OM often causes RT interruption and consequently interferes with RT fraction regimen [30]. The decreased local control rate and reduced survival are the worse consequence of discontinuation of RT [31, 32]. LLLT is a widely used alternative to OM treatment [11, 20, 22, 23]. There are a vast number of LLLT protocols to treat OM [24]. But little studies combine two LLLT wavelengths [21]. Moreover, there are no precise highly reproducible and predictable protocols to repair wounds [33]. Evidence suggests that red and infrared combination at the same dose (20 J/cm^2) brings best results in clinical and histological in the recovery of wounds [34]. Also, it was demonstrated that infrared and red LLLT collaborates to tissue repair and pain control [33, 35, 36]. Considering evidence related to the benefits in a combination of red and infrared LLLT in the

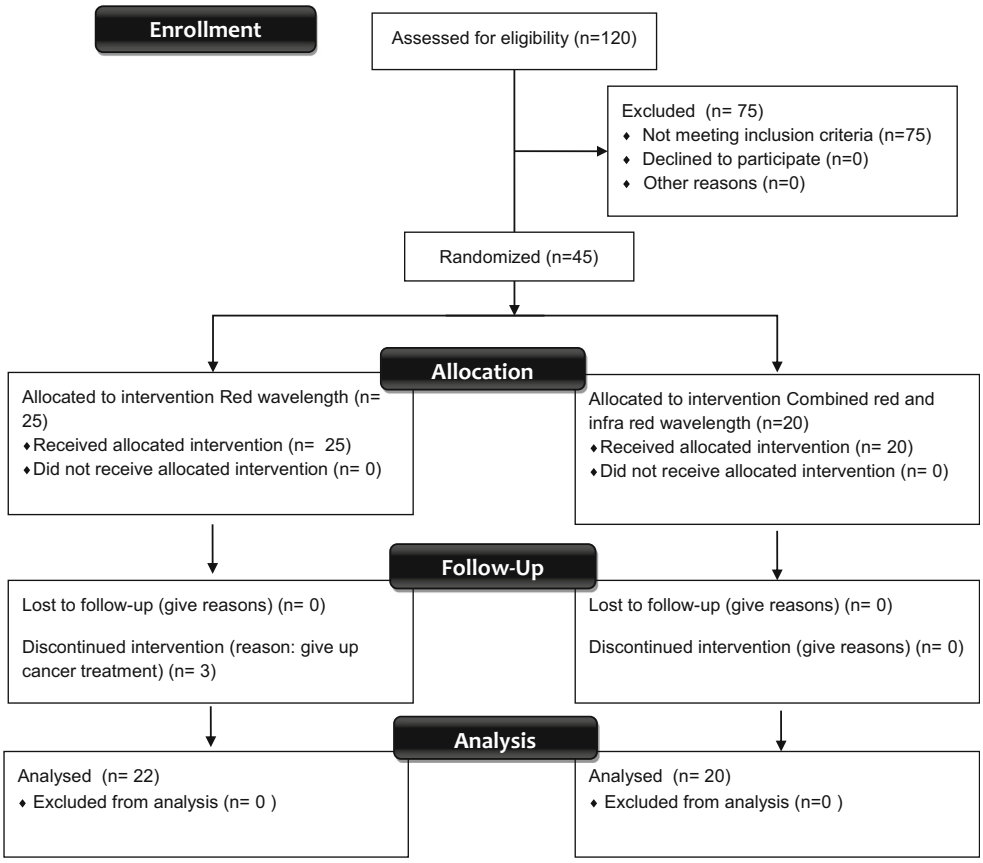

Fig. 2 Flow diagram

Table 1 Comparison of clinical characteristics between groups 1 and 2

	Group 1		Group 2		
	N	%	*N*	%	*p* value
Treatment					
RT	2	66.70	1	33.30	
RT + CT	12	50.00	12	50.00	
RT + CT + SUR	4	80.00	1	20.00	
RT + SUR	2	20.00	8	80.00	0.130
Chemotherapy scheme					
Cisplatin	12	52.17	11	47.83	
Cisplatin and Fluorouracil	3	60.00	2	40.00	
Cisplatin and Gemzar	1	100.00	0	0.00	0.380
Radiation therapy dose					
Igual of less than 64 Gy	7	70.00	3	30.00	
More than 64 Gy	13	40.60	19	59.40	0.104
Primary tumor (T)					
T1	1	33.30	2	66.70	
T2	6	46.20	7	53.80	
T3	4	57.10	3	42.90	
T4	8	44.40	10	55.60	
TX	0	0.00	0	0.00	0.906
Regional lymph nodes (N)					
N0	4	57.10	3	42.90	
N1	5	41.70	7	58.30	
N2	6	42.90	8	57.10	
N3	4	57.10	3	42.90	
NX	1	50.00	1	50.00	0.728
Distant metastasis (M)					
N0	4	50.00	4	50.00	
N1	1	33.34	2	66.66	
NX	15	48.38	16	52.62	0.712
Anatomical site					
Oral cavity	18	48.60	19	51.40	
Oropharynx	1	25.00	3	75.00	
Hypopharynx	1	100.00	0	0.00	0.380
Sex					
Male	16	44.40	20	55.60	
Female	4	66.70	2	33.30	0.286
Age (years)					
Range	35–82		42–86		
Mean (SD)	58.05	(12.44)	61.82	(10.96)	0.801

In asterisk, significant *p* values. *RT* radiation therapy, *CT* chemotherapy, *SUR* surgery. No differences in clinical features between groups 1 and 2 were observed

current study, a new LLLT protocol specifically for OM treatment was proposed. But it is important to highlight that wavelength, energy, energy density, original power, power density, and frequency are all necessary parameters that need to be adjusted to replicate a successful treatment [37].

Table 2 Comparison of oral mucositis grade between groups 1 and 2

	Group 1		Group 2		
Mucositis grade	*N*	%	*N*	%	*p* value
0	3	50.00	3	50.00	
1	5	100.00	0	0.00	
2	8	30.80	18	69.20	
3	3	75.00	1	25.00	
4	1	100.00	0	0.00	*0.029**
Mucositis grade					
1	5	100.00	0	0.00	
2, 3, 4	12	38.70	19	61.30	*0.016**

* significant *p* values. Group 1 showed a reduction of the worse mucositis grade in comparison to group 2

In the current study, preclinical research was performed to establish and adjust the LLLT parameters. The preclinical analyses demonstrated that total energy of 9 J of both 660- and 808-nm wavelengths (100 mW, spot size 3 mm^2, and dose 300 J/cm^2) for point stimulates tissue repair. Additionally, the preclinical study also confirmed that LLLT promotes fibroblast proliferation [38]. Based on preclinical data, the safety and efficiency of the protocol were tested in a controlled study which compared two groups. Group 1 (both 660- and 808-nm wavelengths, 9 J of total energy, 100 mW, spot size 3 mm^2, the total dose delivered to oral mucosa was 300 J/cm^2 twice a week) presented reduction of OM worse grade and analgesics prescription in comparison to group 2 (660-nm wavelength, 9 J of total energy, 100 mW, spot size 3 mm^2, the total dose delivered to oral mucosa was 300 J/cm^2 twice a week). The trial limitations are the small numbers of participants and the data collection in only one study center.

The energy applied in the LLLT in the current study is two times higher than a previous recommendation [39]. Another critical difference between the current protocol is the number of sessions which is only twice a week. It is essential to highlight that the weekday selection (Tuesdays and Thursdays) is crucial. The rationale for the current protocol was based on the

Table 3 Comparison of pain symptoms between groups 1 and 2

Analgesics prescription	Group 1		Group 2		*p* value
	N	%	*N*	%	
Yes	4	26.70	11	73.30	
No	16	59.30	11	40.70	*0.043**
Worse pain in VAS					
Score < 9	14	58.30	10	41.70	
Score ≥ 9	6	33.30	12	66.70	0.098

* significant *p* values. Group 1 significantly reduced analgesics prescription in comparison to group 2. No differences between groups 1 and 2 were observed in worse pain observed in the visual analog scale for pain

fact that most OSCC patients are not treated with RT on the weekends [40]. So on Mondays, fibroblasts had 48 h to recover from RT. On Tuesdays, there was a necessity of fibroblast stimulation because of the RT received on Mondays. According to our in vitro results, LLLT increase fibroblast proliferation for 24 h. On the other hand, fibroblast proliferation is critically decreased in an energy-dependent manner after 48 and 72 h after LLLT [41] which highlighted the necessity of LLLT on Thursdays. So, LLLT on Tuesdays and Thursdays can maintain fibroblast recovery during the week.

In the current study, a combination of LLLT wavelengths reduced OM worse grade. The in vitro experiments suggested that for a combination of wavelengths, a higher dose is more efficient for fibroblast proliferation. Even with exciting results presented in the current study, there are difficulties in understanding the molecular wound healing mechanism in irradiated OSCC patients because the effects of the combination of RT and LLLT are unclear [42]. Additionally, new RT techniques in oral mucosa are described and need investigation [43]. Taken all the facts together, the preclinical and clinical data of the current study corroborates that the combination of red and infrared wavelengths at a higher dose (300 J/cm^2) reduced OM grade in comparison to the use of only red and infrared wavelengths (3 J/cm^2) or the exclusive use of red wavelength.

It was also observed that the combination of red and infrared at a higher dose (300 J/cm^2) reduced analgesics prescription. It is important to highlight that doctors and patients were blinded about LLLT. On the other hand, wavelength combination in LLLT did not present a VAS reduction. The treatment decision is based on patients' signals and symptoms while VAS only focuses on the patients' perception [44]. The presence of pain in cancer patients, especially during OSCC treatment, needs attention [45] even considering the recent technological benefits from RT [46]. Infrared LLLT acts in the cell membrane thereby changing essential transport mechanisms such as the sodium-potassium pump [14, 19], and consequently reduce pain [47]. Also, LLLT with infrared light (808 nm) could also reduce inflammatory mediators related to pain sensation such as interleukins 1 and 6 which are associated with pain [25, 48].

In conclusion, the current study demonstrated that a combination of red and infrared at a higher dose (300 J/cm^2) reduced both oral mucositis grade and analgesics prescription. The effects of the combination of RT and LLLT are unclear and need more studies.

Acknowledgements Dr. Guimarães, Dr. Dr. Santos, and Dr. de Paula are research fellows of the CNPq. Dr. Farias is a research fellow of FAPEMIG.

Funding information This study was supported by grants from the Conselho Nacional de Desenvolvimento Científico e Tecnológico (CNPq), Coordenação de Aperfeiçoamento de Pessoal de Nível Superior (CAPES), and the Fundação de Amparo a Pesquisa do Estado de Minas Gerais (FAPEMIG).

Compliance with ethical standards

Conflict of interest The authors declare that they have no conflicts of interest.

Ethical approval Ethical approval for this study was obtained from the relevant Institutional Review Board (48215415.0.0000.5146). The study was also registered in National Clinical Trials (UTN: U1111-1177-9023/67hjmn).

Informed consent All patients signed informed consent.

References

1. Ferlay J, Soerjomataram I, Dikshit R, Eser S, Mathers C, Rebelo M et al (2015) Cancer incidence and mortality worldwide: sources, methods and major patterns in GLOBOCAN 2012. Int J Cancer 136(5):E359–E386
2. Warnakulasuriya S (2009) Global epidemiology of oral and oropharyngeal cancer. Oral Oncol 45(4–5):309–316
3. Stelzle F, Knipfer C, Schuster M, Bocklet T, Noth E, Adler W et al (2013) Factors influencing relative speech intelligibility in patients with oral squamous cell carcinoma: a prospective study using automatic, computer-based speech analysis. Int J Oral Maxillofac Surg 42(11):1377–1384
4. Speksnijder CM, van der Glas HW, van der Bilt A, van Es RJ, van der Rijt E, Koole R (2010) Oral function after oncological intervention in the oral cavity: a retrospective study. J Oral Maxillofac Surg 68(6):1231–1237
5. Maria OM, Eliopoulos N, Muanza T (2017) Radiation-induced oral mucositis. Front Oncol 7:89
6. Sonis ST, Elting LS, Keefe D, Peterson DE, Schubert M, Hauer-Jensen M et al (2004) Perspectives on cancer therapy-induced mucosal injury: pathogenesis, measurement, epidemiology, and consequences for patients. Cancer 100(9 Suppl):1995–2025
7. Riley P, Glenny AM, Worthington HV, Littlewood A, Clarkson JE, McCabe MG (2015) Interventions for preventing oral mucositis in patients with cancer receiving treatment: oral cryotherapy. Cochrane Database Syst Rev (12):CD011552
8. von Bultzingslowen I, Brennan MT, Spijkervet FK, Logan R, Stringer A, Raber-Durlacher JE et al (2006) Growth factors and cytokines in the prevention and treatment of oral and gastrointestinal mucositis. Support Care Cancer 14(6):519–527
9. Rastogi M, Khurana R, Revannasiddaiah S, Jaiswal I, Nanda SS, Gupta P et al (2017) Role of benzydamine hydrochloride in the prevention of oral mucositis in head and neck cancer patients treated with radiotherapy (>50 Gy) with or without chemotherapy. Support Care Cancer 25(5):1439–1443
10. Peterson DE, Boers-Doets CB, Bensadoun RJ, Herrstedt J, Committee EG (2015) Management of oral and gastrointestinal mucosal injury: ESMO Clinical Practice Guidelines for diagnosis, treatment, and follow-up. Ann Oncol 26(Suppl 5):v139–v151
11. Carvalho PA, Jaguar GC, Pellizzon AC, Prado JD, Lopes RN, Alves FA (2011) Evaluation of low-level laser therapy in the prevention and treatment of radiation-induced mucositis: a double-blind randomized study in head and neck cancer patients. Oral Oncol 47(12):1176–1181
12. Elad S, Zadik Y (2016) Chronic oral mucositis after radiotherapy to the head and neck: a new insight. Support Care Cancer 24(11): 4825–4830

13. Calderhead RG, Vasily DB (2016) Low level light therapy with light-emitting diodes for the aging face. Clin Plast Surg 43(3): 541–550
14. Kim WS, Calderhead RG (2011) Is light-emitting diode phototherapy (LED-LLLT) really effective? Laser Ther 20(3):205–215
15. Huang YY, Sharma SK, Carroll J, Hamblin MR (2011) Biphasic dose response in low level light therapy—an update. Dose-Response 9(4):602–618
16. Buravlev EA, Zhidkova TV, Osipov AN, Vladimirov YA (2015) Are the mitochondrial respiratory complexes blocked by NO the targets for the laser and LED therapy? Lasers Med Sci 30(1):173–180
17. Hahm E, Kulhari S, Arany Praveen R (2012) Targeting the pain, inflammation and immune (PII) axis: plausible rationale for LLLT. Photonics Lasers Med 241
18. Niemz MH (2007) Laser-Tissue Interactions. 308 p
19. Karu T (1999) Primary and secondary mechanisms of action of visible to near-IR radiation on cells. J Photochem Photobiol B 49(1):1–17
20. Gouvea de Lima A, Villar RC, de Castro G Jr, Antequera R, Gil E, Rosalmeida MC et al (2012) Oral mucositis prevention by low-level laser therapy in head-and-neck cancer patients undergoing concurrent chemoradiotherapy: a phase III randomized study. Int J Radiat Oncol Biol Phys 82(1):270–275
21. Simoes A, Eduardo FP, Luiz AC, Campos L, Sa PH, Cristofaro M et al (2009) Laser phototherapy as topical prophylaxis against head and neck cancer radiotherapy-induced oral mucositis: comparison between low and high/low power lasers. Lasers Surg Med 41(4): 264–270
22. Campos L, Simoes A, Sa PH, Eduardo Cde P (2009) Improvement in quality of life of an oncological patient by laser phototherapy. Photomed Laser Surg 27(2):371–374
23. Migliorati C, Hewson I, Lalla RV, Antunes HS, Estilo CL, Hodgson B et al (2013) Systematic review of laser and other light therapy for the management of oral mucositis in cancer patients. Support Care Cancer 21(1):333–341
24. Bjordal JM, Bensadoun RJ, Tuner J, Frigo L, Gjerde K, Lopes-Martins RA (2011) A systematic review with meta-analysis of the effect of low-level laser therapy (LLLT) in cancer therapy-induced oral mucositis. Support Care Cancer 19(8):1069–1077
25. Coluzzi DJ, Parker SPA (2017) Lasers in dentistry current concepts. 400 p
26. Zhou Y, Fan W, Xiao Y (2014) The effect of hypoxia on the stemness and differentiation capacity of PDLC and DPC. Biomed Res Int 2014:890675
27. Schneider CA, Rasband WS, Eliceiri KW (2012) NIH image to ImageJ: 25 years of image analysis. Nat Methods 9(7):671–675
28. Rosner B (2011) Fundamentals of biostatistics. Brooks/Cole, Cengage Learning, Boston
29. Rieger JM, Zalmanowitz JG, Wolfaardt JF (2006) Functional outcomes after organ preservation treatment in head and neck cancer: a critical review of the literature. Int J Oral Maxillofac Surg 35(7): 581–587
30. Biswal BM (2008) Current trends in the management of oral mucositis related to cancer treatment. Malays J Med Sci 15(3):4–13
31. Cox JD, Pajak TF, Marcial VA, Coia L, Mohiuddin M, Fu KK et al (1992) Interruptions adversely affect local control and survival with hyperfractionated radiation therapy of carcinomas of the upper respiratory and digestive tracts. New evidence for accelerated proliferation from Radiation Therapy Oncology Group Protocol 8313. Cancer 69(11):2744–2748
32. Peters LJ, O'Sullivan B, Giralt J, Fitzgerald TJ, Trotti A, Bernier J et al (2010) Critical impact of radiotherapy protocol compliance and quality in the treatment of advanced head and neck cancer: results from TROG 02.02. J Clin Oncol 28(18):2996–3001
33. de Lima FJ, Barbosa FT, de Sousa-Rodrigues CF (2014) Use alone or in combination of red and infrared laser in skin wounds. J Lasers Med Sci 5(2):51–57
34. Mendez TM, Pinheiro AL, Pacheco MT, Nascimento PM, Ramalho LM (2004) Dose and wavelength of laser light have influence on the repair of cutaneous wounds. J Clin Laser Med Surg 22(1):19–25
35. Enwemeka CS, Parker JC, Dowdy DS, Harkness EE, Sanford LE, Woodruff LD (2004) The efficacy of low-power lasers in tissue repair and pain control: a meta-analysis study. Photomed Laser Surg 22(4):323–329
36. Woodruff LD, Bounkeo JM, Brannon WM, Dawes KS, Barham CD, Waddell DL et al (2004) The efficacy of laser therapy in wound repair: a meta-analysis of the literature. Photomed Laser Surg 22(3): 241–247
37. Zecha JA, Raber-Durlacher JE, Nair RG, Epstein JB, Sonis ST, Elad S et al (2016) Low level laser therapy/photobiomodulation in the management of side effects of chemoradiation therapy in head and neck cancer: part 1: mechanisms of action, dosimetric, and safety considerations. Support Care Cancer 24(6):2781–2792
38. Schartinger VH, Galvan O, Riechelmann H, Dudas J (2012) Differential responses of fibroblasts, non-neoplastic epithelial cells, and oral carcinoma cells to low-level laser therapy. Support Care Cancer 20(3):523–529
39. Zecha JA, Raber-Durlacher JE, Nair RG, Epstein JB, Elad S, Hamblin MR et al (2016) Low-level laser therapy/photobiomodulation in the management of side effects of chemoradiation therapy in head and neck cancer: part 2: proposed applications and treatment protocols. Support Care Cancer 24(6):2793–2805
40. Yeo R, Campbell T, Fairchild A (2012) Is weekend radiation therapy always justified? J Med Imaging Radiat Sci 43(1):38–42
41. Kreisler M, Christoffers AB, Al-Haj H, Willershausen B, d'Hoedt B (2002) Low level 809-nm diode laser-induced in vitro stimulation of the proliferation of human gingival fibroblasts. Lasers Surg Med 30(5):365–369
42. Haubner F, Ohmann E, Pohl F, Strutz J, Gassner HG (2012) Wound healing after radiation therapy: review of the literature. Radiat Oncol 7:162
43. Sanguineti G, Endres EJ, Gunn BG, Parker B (2006) Is there a "mucosa-sparing" benefit of IMRT for head-and-neck cancer? Int J Radiat Oncol Biol Phys 66(3):931–938
44. Wewers ME, Lowe NK (1990) A critical review of visual analogue scales in the measurement of clinical phenomena. Res Nurs Health 13(4):227–236
45. Schaller A, Dragioti E, Liedberg GM, Larsson B (2017) Quality of life during early radiotherapy in patients with head and neck cancer and pain. J Pain Res 10:1697–1704
46. Wan Leung S, Lee TF, Chien CY, Chao PJ, Tsai WL, Fang FM (2011) Health-related quality of life in 640 head and neck cancer survivors after radiotherapy using EORTC QLQ-C30 and QLQ-H&N35 questionnaires. BMC Cancer 11:128
47. Lopatina EV, Yachnev IL, Penniyaynen VA, Plakhova VB, Podzorova SA, Shelykh TN et al (2012) Modulation of signal-transducing function of neuronal membrane Na+,K+-ATPase by endogenous ouabain and low-power infrared radiation leads to pain relief. Med Chem 8(1):33–39
48. Alves AC, Vieira R, Leal-Junior E, dos Santos S, Ligeiro AP, Albertini R et al (2013) Effect of low-level laser therapy on the expression of inflammatory mediators and on neutrophils and macrophages in acute joint inflammation. Arthritis Research & Therapy 15(5):R116