

Effect of Low-Level Laser Therapy (660nm) with Output Power of 30 mW in the Management of the Experimentally Induced Oral Mucositis

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Abstract: *The aim of this study was to investigate the effect of 660nm (30mw) low level laser therapy (LLLT) in the management of oral mucositis induced by 60mg/kg MTX (MTX; Pfizer, Italy) in DA. Rat. Thirty-eight rats were included and divided into two groups: (n=16) Control group (no treatment); (n=22) Therapeutic laser 30mw group [LLLT from day 6 to day11] (LLLT from D 6 to D 11). The animals received a intraperitoneal injection of MTX on Days 0 and 3 (30mg/kg). Their radiation parameters were: indium-gallium-aluminum-phosphide (InGaAlP) diode laser PhotonlaseIR model (DMCEQUIPAMENTOS LTDA) (660 nm, 30 mW), beam area of 0.036 cm², 1.16 W/cm², 10 J/cm², power density applied daily of 40.6 J/cm², in contact mode (seven points and five seconds per point) and, one application per day. The experiment lasted 11 days and OM was analyzed by specific clinical scales on days 6 and 11. The animals were sacrificed on Day 11. The laser therapeutic group had statistically significant lower clinical scores than the control group at Day 11. This study showed that positive effects on oral mucositis management were obtained when LLLT was applied in this therapeutic protocol (from D6 to D11). Regarding clinical scores, there were no statistical significant differences between study groups (p<0.05) at day six. In comparison with clinical scores at day six: there were statistically significant negative correlations (R=-0.055) (p=0.0089). According to the methodology used and the results obtained in the present study, LLLT 660nm 30 therapy was the best choice to decrease the severity of OM treatment of experimental oral mucositis with 10J/cm² dose intensity.*

Keywords: Experimental oral mucositis, low level laser therapy, methotrexate, dark agouti rat

1. Introduction

Oral mucositis is a difficulty resulting after radiotherapy and/or cytotoxic for cancer treatment (1). Clinically, mucositis is classified in stages shifting from minimal signs of erythematous abrasions to basic ulceration and ache, which may require the cancer medication to be ended, so leading to a drop in value of life and/or patient life (1-2). Moreover, severe mucositis may lead to prolonged hospital stays and requires special care, such as the intravenous infusion of barbiturates or other drugs and parenteral nutrition, leading to a significant increase in hospital costs. The treatment is generally palliative, aimed at restriction symptoms and controlling infection and bleeding. The most common types of treatment for oral mucositis are topical antimicrobial agents, cytokines to stimulate the medulla, vitamins, growth factors, corticoid and non-alcoholic mouth washes, supplementary amino acids, cryotherapy, and treatment with laser phototherapy (3-6). Studies on the effects of LLLT, either alone or in combination with other therapies, conducted in patients with immunosuppression, have shown that LLLT promotes a reduction in pain intensity and mucositis severity, acceleration of wound healing, analgesic effects and an increase in the salivary flow rate, with no side effects (3, 7). Studies involving animal models allow a better understanding of the pathobiology of mucositis and a more in-depth evaluation of the effects of therapies for the prevention and treatment of this disease. The model for induced mucositis in hamsters was initially proposed by Sonis *et al.* (19) and has proven useful in preclinical studies on new treatment options for mucositis. This model was

applied in a few studies for analyzing the effects of LLLT in the therapeutics of oral mucositis, with positive findings (13-15). Although these studies have obtained promising outcomes, the effects of this protocol have not been. Thus, the aim of the present study was to analyze the effect of LLLT in treating oral mucositis induced by methotrexate (MTX) in rats.

2. Materials and Methods

Thirty-eight male dark agouti rats, weighing 220-280 gm were used in this study and the animals were kept under a standard laboratory conditions and maintained on a 12-hour light/dark cycle at 20 ± 5°C, fed with a standard rat chow and supplied with tap water for drinking.

3. Study Design

Thirty-eight of dark agouti rat (8 weeks of life; body weight: around 220-280 g) were used in this study. The animals were held in reserve in groups of twelve per wire-bottomed cages, with food and water supply.

The animals were randomly divided into two groups:

Group I—induced o.m with MTX (control group) were (16) rats included with no laser therapy.

Group II—induced o.m with MTX (22) rats were included & treated with 30mw (LLLT 30 mw).

The MTX 30 mg/kg was administered to each animal intraperitoneally on Day 0 and 30 mg/Kg was

administered on Day 3. The typical clinical signs of drug side effect, such as a decreased food intake, weight loss, and diarrhea, were watched in methotrexate treated rats from the first day until the eleventh day of the experiment.

The MTX 30 mg/kg was administered to each animal intraperitoneally on Day 0 and 3.

Laser Protocol (LLLT)

Photolase (I) diode laser 660nm LLLT administered as the following:

Twenty-two animals from therapeutic received laser irradiation. The laser parameters were kept ($\lambda = 660$ nm, output power 30 mw, total energy density 10 J/cm^2 , (seven points and five seconds per point) and contact mode as illustrated in figure 1, one application per day. Beam area of 0.036 cm^2 , 30 mW, 1.16 W/cm^2 , 10 J/cm^2 , power density applied daily of 40.6 J/cm^2 .

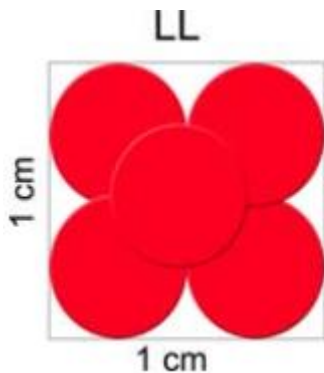


Figure 1: arrangement of different irradiations (LLLT) in the demarcated areas (1 cm^2)

Clinical Evaluation

The clinical aspect of the oral mucosa was observed by one calibrated examiner daily, and the degree of OM was evaluated by specific assessment scale: criteria proposed by World Health Organization (WHO) modified for animals table 1 (24). Table 1 Scoring scale used to grade mucositis in animal using outcomes that are analogous to clinical scoring.

a

Score:	Description:
0	Pouch completely healthy. No erythema or vasodilation.
1	Light to severe erythema and vasodilation. No erosion of mucosa.
2	Severe erythema and vasodilation. Erosion of superficial aspects of mucosa leaving denuded areas. Decreased stippling of mucosa.
3	Formation of off-white ulcers in one or more places. Ulcers may have a yellow/gray due to pseudomembrane. Cumulative size of ulcers should equal about 1/4 of the pouch. Severe erythema and vasodilation.
4	Cumulative size of ulcers should equal about 1/2 of the pouch. Loss of pliability. Severe erythema and vasodilation.
5	Virtually all of pouch is ulcerated. Loss of pliability (pouch can only partially be extracted from mouth).

b

Ulcerated Area ^a		Severity of Erythema ^a		
		None	Not severe	Severe
None	0	0	1	2
< 4mm ²	1	1	2	3
4 – 9mm ²	2	2	3	4
> 9mm ²	3	3	4	5

Statistical Analysis:

All data were analyzed using SPSS (version 23 software) computer program. The results obtained were subjected to statistical testing using the analysis of variance (students T test) to compare quantitative variables and the, Mann-Whitney test, spearman linear correlation to compare qualitative variables, P value > 0.05.

4. Results

Body weight analysis between study groups were:

The body weights of the study groups have shown statistical non-significant differences at (0 day & 6 day), $p = (0.431 \& 0.173)$, respectively. As listed in table 2.

While the body weights of these groups were displayed statistically significant differences at 11 day $p = (0.016)$. as listed in table 2.

Table 2: Comparative analysis of body weight between experimental groups

No. rats	Study group	Times (days)		
		0	6	11
(16)	Control (G1)	Mean \pm SD 239.06 \pm 21.773	Mean \pm SD 207.81 \pm 20.081	Mean \pm SD 184.75 \pm 19.157
	S. E	5.443	5.020	4.789
(22)	L30 (G2)	226.82 \pm 24.424	206.68 \pm 24.257	197.50 \pm 41.341
	S. E	5.207	5.172	5.314
P. value		0.431	0.173	0.011

*Significant difference; p value < 0.05; independent sample t test; SD standard deviation

**Clinical evaluation of experimental oral mucositis
 Clinical scores analysis of the experimentally induced oral mucositis between study groups at day six:**

The results of the experimental induced oral mucositis of the control group G1 clinically showed all sixteen animals had induced oral mucositis as follow: two animals with score one; four animals with score two and ten animals with score three. As shown in table (3) & Fig (2).

regarding therapeutic laser 30 mw group G2, all of twenty-two animals have been induced oral mucositis with the following frequency: three animals with score one; four animals with score two and fifteen animals with score three as shown in table (3).

The therapeutic laser 30 mw and control group G1 showed statistically non-significant differences when compared at day six as listed in table (3).

Table 3: Clinical scores analysis of experimental oral mucositis among study groups at day six

No. rats	Study group	Clinical scoring	%	P* value
N 16	G1 (nontreated)	SCOR1 (2) rats	12.5%	(0.445)
Mean of scores	2.50			
Std. Error of Mean	0.183			
Median	SCOR2	SCOR2 (4) rats	25 %	19
Std. Deviation	0.730			

Range	2			
Minimum	1			
Maximum	3			
		SCOR3 (10) rats	62.5%	
N.22	G2			
Mean	2.55	SCOR1 (3) rats	13.6%	19.86
Std. Error of Mean	0.157			
Median	SCOR3	SCOR2 (4) rats	18.2%	
Std. Deviation	0.739			
Range	2			
Minimum	1	SCOR3 (15) rats	68.2%	
Maximum	3			

*Mann-Whitney test, exact significant

Clinical scores analysis of experimental oral mucositis among study groups at day eleven:

Control group G1 had statistical significant decrease in numbers of the affected rats into two rats with score zero, five rats with score one, four rats with score two & five rats with score three in comparison with the clinical scoring of G1 at day six, as shown in table (3&4). Their negative non-sig correlations in comparison with the scores at day six. (R=-0.171) (p=0.502).

Clinically Laser 30mw group G2 had included twelve rats and became normal with score zero and ten rats had cure to score two, so they had the lowest clinical scores among the study groups, in comparison with clinical scores of G2 at day six, where as 45.5 % of the animals of G2 clinically appeared with normal oral mucosa at day 11, as shown in table (1&2). These group were showed least scores with range (0-1). Statistically significant negative correlations that explained the decrease of clinical scores among the rats group in comparison with their clinical scores at day six (R= -0.204) (p=0.048). The clinical scores exhibited Statistical significant differences (p<0.05) between study groups as shown in table (4).

Table 4: Clinical evaluation of the experimentally induced oral mucositis at day eleven

No. rats	Study group	CLINICAL scoring	%	P* value (G1, G2)	
N 16	G1	SCORE 0 (2) rats	12.5%	(0.000)	
Mean		1.75			
Std. Error of Mean		0.266	SCOR1 (5) rats	31.3%	Mean rank
Median		2			
Std. Deviation		1.065	SCOR2 (4) rats	25.0%	
Range	3			26.94	
Minimum	0				
Maximum	3	SCOR3 (5) rats	31.3%		
N.22	G2	SCORE 0 (12) rats	54.5%		
Mean		0.45			
Std. Error of Mean		0.109			
Median		0	SCOR1 (10) rats	45.5%	14.09
Std. Deviation		0.510			
Range		1	SCOR 0	22.7	

Minimum	0	(5) rats		
Maximum	1			

*, Mann-Whitney test exact significant

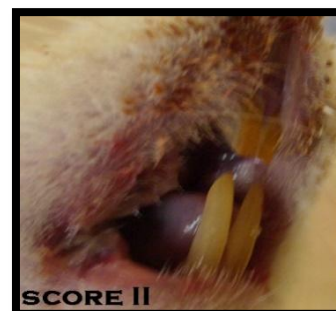


Figure 2: Clinical appearance of experimentally induced oral mucositis between control & therapeutic laser Groups at different time intervals (6 and 11 days)

5. Discussion

Mucositis is a major oncological problem. The entire gastrointestinal and genitourinary tract and also other mucosal surfaces can be affected in recipients of radiotherapy, and/or chemotherapy. Major progress has been made in recent years in understanding the mechanisms of oral and small intestinal mucositis, which appears to be more prominent than colonic damage. This progress is largely due to the development of representative laboratory animal models of mucositis (3). Methotrexate (MTX) is one of the antimetabolic drugs. These drugs act by blocking deoxyribonucleic acid (DNA) and ribonucleic acid (RNA) synthesis, inducing apoptosis and slowing down or stopping cell proliferation in rapidly proliferating cells such as tumors, bone marrow and gut mucosa cells (Soares et al., 2011). Various pharmacological and non-pharmacological agents have tried in preventing and treating oral mucositis. Despite some positive outcomes, it has not been proven to be completely effective in preventing oral mucositis on its own. Till now, there are no single intervention acts on all

phases of oral mucositis (6). Low Level Laser Therapy (LLLT) is a local application of a nonchromatic. However, narrow-band coherent light source used for the photostimulation of biological tissue is recommended as a treatment option for oral mucositis (1, 6). The concept behind the application of laser therapy in the management of oral mucositis is that low power lasers induce anti-inflammatory action and accelerate wound healing by increasing the vascularity and reepithelization. In addition, Lopes *et al.* (20) showed that low power laser appears to decrease the severity of oral mucositis, at least in part, by reducing the COX-2 levels which in turn affect the pathophysiology of oral mucositis (32). Moreover, recent publication from the Multinational Association for Supportive Care in Cancer (MASCC) and the International Society of Oral Oncology (ISOO), recommended the administration of LLLT in patients receiving HSCT, conditioned with high dose chemotherapy with or without total body irradiation (13, 27).

Weight loss could be an indicator of discomfort and pain while eating and drinking (12, 34). Thus, the weight of the animals was determined at baseline, sixth day and eleventh day between the two study groups. The greater weight losses were expected in the Control group. This could be occurred since the animals in the Control group were excluded from daily irradiation by LLLT of their oral mucosa. Logan *et al.*, 2007 have demonstrated that this model can closely mimic the clinical setting. It was possible to characterize the overall health status of the animals measuring their body weight. Animals that received Chemotherapy for several days and after injection of MTX showed less resistance to handling and were clearly debilitated, causing impairment to their feeding abilities. Franca *et al.* & Lopes *et al.* (12, 21) in their literatures were reported an increasing body weight loss in animals that received chemotherapy. However, they could relate this phenomenon first to feeding impairment and second to oral mucositis. It has been detected in their studies that even with improved oral mucositis severity, animals of the experimental groups continued to lose body weight and their food intake did not increase. Thus, it can be assumed that this persistent clinical worsening may be subordinate to destructive effects of cytotoxic drugs. Furthermore Cruz *et al.* (11) were discover probably excessive manipulation could have caused greater stress, which may also explain the weight losses observed in the animals. The present study was indicated significant body weight loss of the control group in comparison with LLLT treated group. While laser group regain some of their weights at the end of this experiment. LLLT using the visible red spectrum has been found to reduce the severity of oral mucositis lesions as well as pain scores (28). Furthermore Cruz *et al.* (8) reported that the LLLT appears to be a simple, non-traumatic technique for the prevention and treatment of radiation induced mucositis. The ability of different molecules (and thus tissues) to absorb LLL energy is known to be dependent on wavelength. Consequently, the shorter wavelengths (632–660nm) have been shown to deposit most of their energy in the superficial layers of irradiated tissues, while longer wavelengths (780–901 nm) will penetrate much deeper.

This characteristic does not appear to be merely a function of absorption. For these reasons, a laser emitting in the visible red (660nm) range was used in the present study, the output power (30mw) of $\lambda=660\text{nm}$ to test possible therapeutic effects. This study investigated clinical features, including the animals weight, performed a clinical analysis of the oral mucosa in which mucositis was induced by 30mg/kg of MTX at day 0, 3.

LLLT has effects only on stressed or diseased tissues. This therapy is able to restore the regular metabolic potential of stressed cells (20). With in this mind, it could be posited that the initial irradiation on an already stressed tissue as occurred in the treated group may have led to a prompt response to the LLLT, whereas the tissue that started to be irradiated when it was in a regular metabolic condition could have been inhibited using a relatively high dose of daily irradiation. The findings of the present study also demonstrate the positive effect of LLLT in reducing the severity of mucositis when this therapeutic protocol was used. On Day 11, following MTX, once an increase in the clinical scores was expected, this group were reduced its initial scores, which were significantly lower than those of the control group at the same period and in comparison, with their scores at day 6.

In other study, the oral mucositis induced by chemotherapy would be resolved after 15 days of low level laser treatment (19-20). Thus, our study revealed something new, i.e., it showed that this therapeutic protocol of LPT presented a positive effect exactly at day eleven, when the clinical signs of oral mucositis are more critical. However lower output power (30mw) exhibited significant positive therapeutic effects with least clinical scores. Similarly, other studies reported the use of high doses of radiation for this purpose, but such radiation has better effects in promoting analgesia (13, 31). Regarding tissue repair, the use of high doses of radiation is related to an apparent delay of the process (7, 20). In contrast, Lara *et al.* (15) were concluded that application of LLLT (GaAIA) on animal model (rats) of mucositis, showed that GaAIA was not effective in comparison to topical dexamethasone treated group.

Unlike the 35 mW laser, the 100 mW laser did not have an effect on the severity of clinical mucositis, indicating a more pronounced anti-inflammatory effect of the lower power laser treatment, through inhibition of COX-2. This reinforces the notion that the laser-use parameters are of paramount importance. In particular, the total time for which irradiation is done could be of importance. During irradiation, *in vivo*, in addition to the local area being targeted, the blood that circulates in this area during exposure also receives irradiation, and the amount of irradiated blood is proportional to the time of irradiation (20). The present data was in counter with Schubert *et al.*, 2007 that were used other treatment parameters, lasers with powers in the range of 50–100 mW would seem to be the most practical to provide appropriate fluency and clinically practical treatment times (28).

6. Conclusion

LLT 660nm, 30mW can be used for treatment of experimental oral mucositis with 10J dose intensity. LLLT 660nm recommended for reduce oral mucositis severity by downregulation of their development clinically.

The irradiated animals were demonstrated enhancement of their daily feeding capability via regaining some of their weight at the end of this experiment.

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Abbreviations

λ wave length
Cox cyclooxygenase
CT chemotherapy
DNA deoxyribonucleic acid
I.P intraperitoneal
Kg kilogram
LLLT low level laser therapy
Mg milligram
MTX methotrexate
mW milliwatt
nm nanometer
OM oral mucositis
RNA ribonucleic acid

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