The role of low level laser therapy on the expression of IL_1 beta in wound healing

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ABSRACT

Background: Low-level laser therapy (LLLT) has been extensively applied to improve wound healing due to some biostimulatory properties presented by laser arrays apparently able to accelerate the repair of soft tissue injuries. However, the role of proinflammatory interlukines not been studied yet. IL_1 β represent one of the most important poroinflammatory interlukines that involved in wound healing. The goal of this study was to investigate the effect of 790-805nm diode laser on the expression of IL_1 β during wound healing in mice.

Materials and Methods: Standard-sized wounds (1.5cm) were carried out in the face of 96 white albino mice. Half of them underwent LLLT treatment (360 J/cm ^{2}) at 790-805 nm delivered immediately after wound procedure. The repairing area was removed and stained with immunohistochemistry technique to detect the expression of IL_1 β.

Results it had been found that LLLT was able to increase the expression of the IL_1 β in early phases of healing as well as to enhance epithelization remodeling process at both 7 th and 14 th days of wound healing.

Conclusions : The LLLT protocol tested in this study resulted in increased the expression of IL_1 β in the lased group significantly at day 7 of healing period which affect wound healing.

Keywords: low level laser therapy (LLLT), photobiomodulation, IL_1β. (J Bagh Coll Dentistry 2013; 25(2):108-113).

INTRODUCTION

LLLT or **"cold"** lasers use radiation intensities so low that it is thought that any biological effects that occur are due to the direct effects of radiation rather than the result of heating. LLLT devices have been advocated for relief of pain, healing of soft tissue disorders, and treatment of peripheral neuropathies and primarily include the gallium-aluminum (GaAL), gallium-arsenide (GaAs), gallium-aluminum-arsenide (GaAlA) and helium-neon (He-Ne) laser. The He-Ne laser was the first laser available and is reported to have beneficial effects in both wound healing and dentistry. The GaAs and GaAlAs laser have been most commonly used for the treatment of pain and inflammation and in lower doses for wound healing as they have deeper tissue penetration than the He-Ne laser [1-4]. Tissue healing (or tissue repair) refers to the body's replacement of destroyed tissue by living tissue and comprises two essential components - Regeneration and Repair. The differentiation between the two is based on the resultant tissue. In regeneration, specialized tissues are replaced by the proliferation of surrounding undamaged specialized cells. In repair, lost tissue is replaced by granulation tissue which matures to form scar tissue.

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There are several different ways to'divide up' the healing process, but the allocation of 4 phases is common and will be adopted here – these being bleeding, inflammation, proliferation and remodeling [5]. Different substances absorb light of different wavelengths for example; the cells of injured skin are more sensitive than those of intact tissue. Once the target cells have absorbed the photons a cascade of biochemical events occur with the ultimate result of accelerated wound healing. Laser therapy is thought to work through a variety of mechanisms:

- Growth factor response within cells and tissue as a result of increased ATP and protein synthesis, improved cell proliferation and change in cell membrane permeability to calcium up-take.
- A cascade of metabolic effects results in various physiological changes which results in improved tissue repair, faster resolution of the inflammatory response, and a reduction in pain [6]. The most effective growth factors and cytokines in cutaneous wound healing are platelet derived growth factor, vascular endothelial growth factor, transforming growth factor, fibroblast growth factor, epidermal growth factor insulin-like growth factor and interlukines [7].

Interleukin -1 beta (IL-β) a member of interlukin 1 cytokine family. This cytokine is produced by activated macrophages as a proprotein which is proteolytically processed to its active form by caspase -1.This cytokine is an important mediator of the inflammatory response and is involved in a variety of cellular activities ;including cell proliferation, differention, and

apoptosis.. The function of IL-1 β could be summarized as follow:

1. Upregulate inflammatory response.
2. Recruit and activate neutrophils monocytes.
3. Cause upregulation of endothelial adhesion molecules.
4. Promote lymphocyte endothelial transmigration [8].

The goal of this study was to investigate the effect of 780-805nm diode laser on the expression of IL-1 β in incisional wound healing in mice.

MATERIALS AND METHODS:

Ninety –six white albino mice weighting 150-200gm; 3-6 months old were used in this study. The animals were divided into 2 groups, control group which includes 48 animals and lased group includes 48 animals.

Laser system: Laser system which is used in this study is an infrared (Ga Al As) diode laser, class IV laser (K-Laser, Italy), its wavelength is 790-805 nm, mode of operation is modulated cw, maximum cw power is 4 W.

Animal irradiation: The surgical field was done on the check side. An incision was done with 1.5cm length. The animals of lased group had been irradiated by an infrared diode laser while the wound of the control group did'nt irradiated. The animals were divided into 4 groups related to healing period intervals:- The specimens were taken from both groups in 1st, 3rd, 7th and 14th days and prepared for histological examination.

Animals of laser experimental groups were treated with ArGaAl laser arrays of 790-805-nm wavelength obtained from a laser apparatus(K-LASER-ITALY). The treatment applied immediately following surgical procedure consisted of 780-905 nm for 90 s, 4W (output power), and an energy density of 360 J/cm ^{2}. Focal spot was 8mm. Laser array was positioned directly over the animal at a vertical distance of 0.5 cm from the edge of the wound and irradiation was performed at one spot to cover the wound area. After the sacrifice of the animals, the wound area was surgically removed, fixed in buffered 10% formalin, and paraffin embedded. Subsequently, serial 4-µm sections were obtained and prepared to be stained immunohistochemistry with LSAB type. The immunostaining kit and the interlukine 1 beta was obtained from Santa Cruz company,USA (Table 1).

Table1: Data information of Santa Cruze IL-1beta

Growth factor	Chromosomal location	Source	Product
IL-1.ß	Il-1bhuman mapping to 2q14'IL-1b mouse mappingto2 F	Rabbit polyclonal antibody raised against amino acids 117-269 of IL-1B of human origin.	Vial contains 200µg in 0.1ml of PBS with<0.1 % sodium azide and 0.1 %gelatin.

Immunohistochemistry staining procedure:

The procedure of immunostaining includes several steps which were: Deparaffinizion the tissue sections, rehydration ,perxidase block ,protein block,primary antibodies,biotinylated link(secondary antibody), streptavidin-HRPreagent,DAB.Counter stain, dehydration, mounting.

Immunohistochemical staining analysis: IL-1ß: the localization of the stain will be extracellular, as pleiotrophic expression. It will release from neutrophils and macrophages and is in the epithelial cells of epidermis and stromal component (inflammatory cells, blood vessels fibrous connective tissue) as brown stain.

The scoring system was used according to [9].Tow-four fields from each section were used and expressed as counts per mm2 area for each animal. Scoring of expression was 0(no stain), 1(<10), 2(10-25%), 3(25-50%), 4(50%<).

Statistical analysis:

Results are expressed as mean ±standard deviation (SD). Statistical difference was assessed by analysis of variance (ANOVA) followed by *t* test .A *P* value<0.05 was considered to be significant; while 0.001 was highly significant.

RESULTS

Control group: 1. Epidermal expression: The IHC positive stain for IL-B expressed in day 1, then increased in day 3 to return decreased 7, and increased again in day 14 on keratinocytes.

2. Stromal expression: the positive stain expressed in day 1 then increased in day 3 to be decreased in day7 and increased again in 14. **(Fig.1, 3, 5 and 7).**

Laser group: 1. Epidermal expression: expressed in day 1 and decreased in day 3, 7, and day 14.

2. Stromal expression: expressed in day 1 and increased in day 3, 7 , and 14. **(Fig.2,4,6,and 8).**

Statistical analysis of IL-1β:

A-Comparison between the control and laser groups: According to **(Table 2), t**here was significant difference between the control and laser groups at day 7 healing period for stromal expression (P≤ 0.05).

B-Comparison between epidermal and stromal expression of IL-1β:

The result of the test **(Table-3)** showed that there was no significant difference between epidermal and stromal expression of IL-1β in control group except in day 1 of healing period (P≤ 0.05); while for laser group there was significant difference (P≤ 0.05) between epidermal and stromal expression of IL-1β in day 1and 3 of healing and significance in day 14 for (P≤ 0.05). Day 7 showed highly significant difference 1(P≤ 0.001).

DISCUSSION

The healing process is initiated immediately after injury by the release of various growth factors, cytokines and low-molecular weight compounds from the injured blood vessels and from the degranulating platelets [10]. The inflammatory cells start infiltrating the wound tissue within hours after the injury and represent a major source of growth factors like PDGF,VEGF,TGF, and cytokines likeIL-1,IL-2,IL-3,IL-4,IL-6 andIL-8 ;which initiate the proliferative phase of wound repair which starts with the migration and proliferation of keratinocytes at the wound edge which is followed by proliferation of dermal fibroblasts in neighborhood of the wound to form a framework of fibrous connective tissue connecting wound edges together to closed the wound gap and allow completion of healing[7].Low level laser therapy affect different types of cells and tissue as a result of increased ATP production and protein synthesis within the cells which affect cells proliferation and change in cell membrane permeability to increase calcium up-take. These factors are produced by immune cells infiltrated to the wound area like (neutrophils, monocytes, macrophages and lymphocyte, in addition to peripheral nerve endings, fibroblasts, endothelial cells and other non-immune cells [10]. Il-1β represents a proliferation cytokines that regulates many aspects of the immune and inflammatory responses. There are 2 types of IL-1ligands with agonist activity; like IL-1α and IL-β which are produced by various kinds of cells such as neutrophils, monocytes, macrophages, fibroblasts and keratinocytes. Both IL-1α and IL-β bind to same receptors and have similar ; if not identical ,biological properties which their expression more strongly enhanced during wound healing [11] .It's the first important cytokines that released after injury and it's responsible for initiation of inflammation, cell recruitment to wound bed, debris removal and promotion of proliferative phase of healing [10] .In this study; the epidermal expression of IL-1ß in the control group was seen in the day 3 higher than day 1 .This result could be explained from point of view that the lymphocytes infiltrate the epidermis at wound site and adhere to keratinocytes which lead to activation of both cell types to generate pro-inflammatory cytokines including IL-1ß and when the acute inflammation subsided in day 7 the expression of epidermal IL-1β inclined in its lowest level may be due to reduction of pro-inflammatory effect need [12] .The elevation of epidermal expression of IL-1ß in day 14 again may be related to their apoptotic effect of this cytokine [13,14].The stromal expression of IL-1ß in the control group was also seen in higher level in the day 1 then decline in day 3 and 7. This may be due to the pro-inflammatory effect of IL-1ß and its relation with neutrophils count which are represented one of the major producers of this cytokine [11] .The elevation of both epidermal and stromal expression of IL-1ß in the control group in day 14 may be not related to its pro-inflammatory effect but due to its apoptosis effect on keratinocytes to affect apoptosis of these cells during remodeling phase of wound healing and the cell responsible for this is macrophage [13,14] . This result was confirmed by comparison of the IL-1ß expression between epidermis and stroma in day 1 which was significant may be due to the neutrophils' count in acute inflammatory phase of wound healing [11] . The epidermal expression of IL-1ß in the laser group was seen in day 1 and decreased gradually in day 3,7 and 14. This may be due to the role of LLLT on the surface epithelium cells (keratinocytes) to produce the por-inflammatory cytokines including IL-1ß which is needed in the acute inflammation during wound healing [11] , but this effect reduced later on may be due to LLLT anti-inflammatory effects are directly related to reduction of pro-inflammatory cytokines, as well as the amount of chemical mediators. The results of indicate that LLLT induces an inflammatory reaction that may modulate transcription factors linked to mRNA expression proinflammatory cytokines. These data are corroborated by previous studies which suggested that laser therapy can reduce the production of inflammatory mediators and events that contribute to the inhibition of IL-1*β*.The stromal expression of IL-1ß in the laser group was seen in day 1 and decrease in day 3.This early

elevation in the expression may be due to the effect of LLLT on neutrophils and lymphocytes to produce pro-inflammatory cytokines including IL-1ß in acute inflammation phase of wound healing which subside in day 3 of healing period [7] while the elevation in the stromal expression in day 7and 14 may be due to that LLLT stimulated keratinocytes production of IL-1ß and which could be affect wound healing by promotion of proliferative phase of healing [15,11] .The comparison showed that the significant difference between the control and laser group only seen in stromal expression at day 7 of healing period. This may be due to the activated macrophages and fibroblasts production of IL-1ß that play a role in cell proliferation and collagen matrix deposition , pro-matrix metalloproteinase during wound healing [16-19] . This agreed with [20] who found that IL-1 concentration in burn tissue wound would reach its peak at day 6 then declined gradually which indicate correlation with earlier phases of healing, but disagreed with [21] who found that gene expression of IL-1beta and IFN-gamma was significantly and suggested that LLLT decreases the amount of inflammation and accelerates the wound healing process, altering the expression of genes responsible for the production of inflammatory cytokines.As a general observation; IL-1ß seemed to affect wound healing significantly and affected by LLLT which applied immediately post-wounding.

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Table 2: Independent t-test for IL-1B expression for the significant mean difference between control and laser groups

Day	control		Laser		p-value	t-test	Sig.
	M	SD	M	SD			
1-epidermal	.666	0.01	.833	.38	.377	-.919	NS
1-stromal	1	.426	1.33	.65	.153	-1.83	NS
3-epidermal	.75	.45	.58	.514	.418	.842	NS
3-stromal	.91	.666	.58	.79	.589	.842	NS
7-epidermal	.25	.45	.58	.51	.120	-1.684	NS
7-stromal	.33	.65	1.08	.666	.017	-2.783	**S**
14-epidermal	.666	.49	.58	.51	.693	.405	NS
14-stromal	.75	.62	1	.73	.389	-.897	NS

Table 3: Paired t-test for epidermal and stromal expression of IL-1β in control and laser group at different healing period

Period	Mean difference	SE	T-test	P-VALUE	SIG.
Day-1/control	-.33	.142	2.345	.038	**S**
Day-1/laser	-.42	.183	2.159	.053	S
Day-3/control	-.17	.167	1	.338	NS
Day-3/laser	-.5	.23	2.171	.52	S
Day-7/control	-.08	.083	1	.338	NS
Day-7/laser	-.5	.151	3.317	.007	**HS**
Day-14/control	-.08	-.083	1	.338	NS
Day-14/laser	-.42	.149	2.803	.0172	**S**

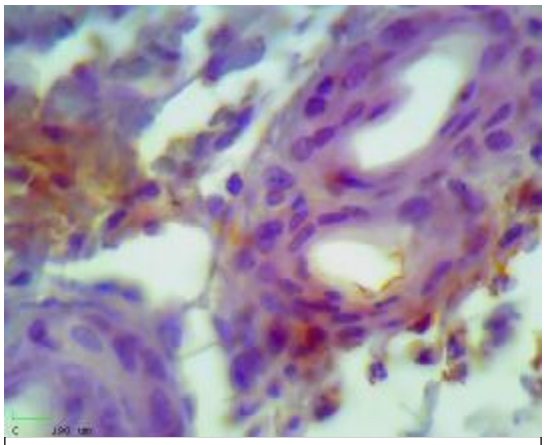

Fig 1: Day 1 control group showing stromal expression of IL-1β

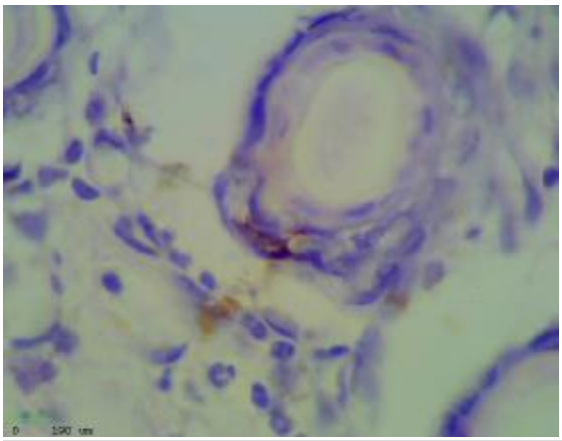

Fig 2: Day 1 laser group showing stromal expression of IL-1β

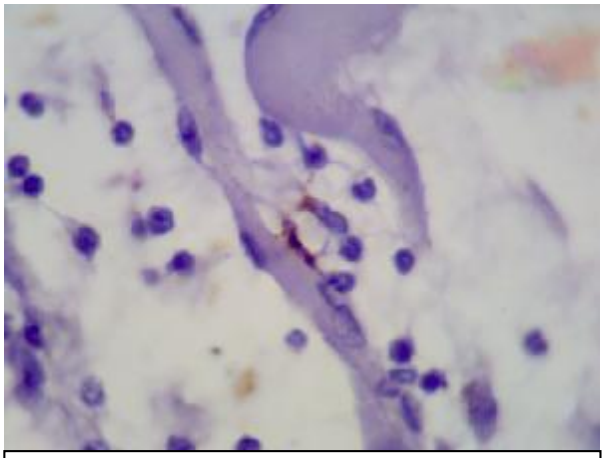

Fig 3: Day 3 control group showing stromal expression of IL-1β

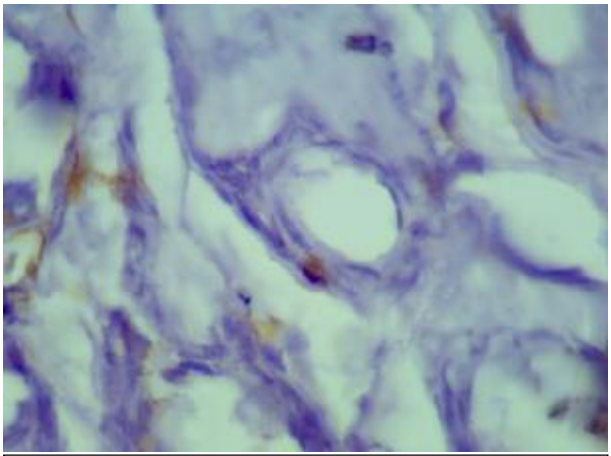

Fig 4: Day 3 lased group showing stromal expression of IL-1β

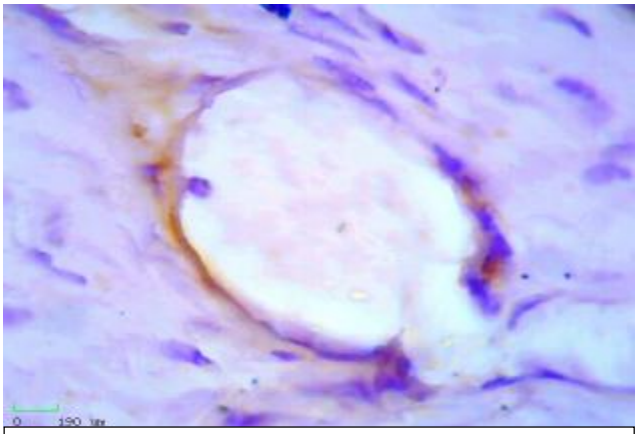

Fig 5: Day 7 control group showing stromal expression of IL-1β

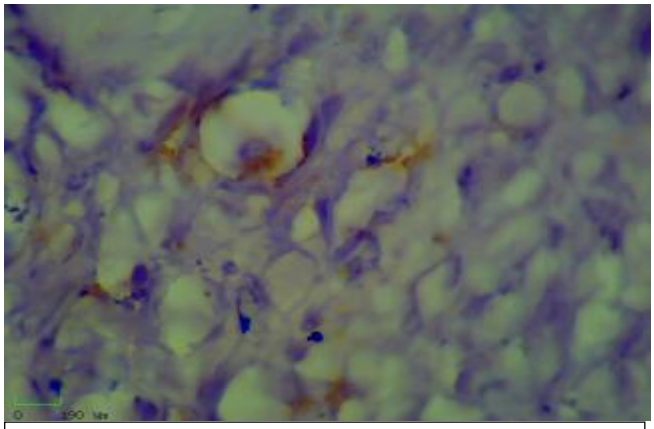

Fig 6: Day 7 lased group showing stromal expression of IL-1β

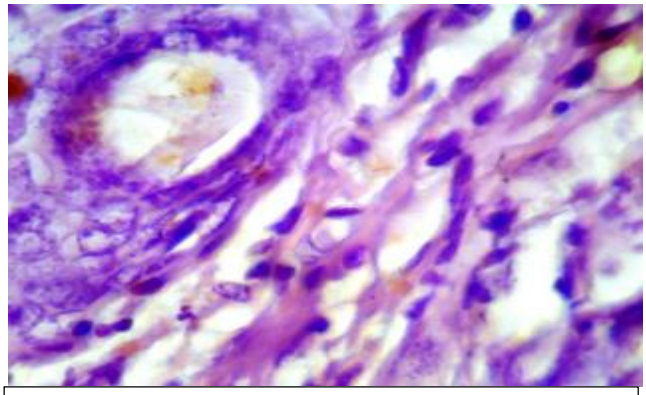

Fig 7: Day 14 control group showing stromal expression of IL-1β

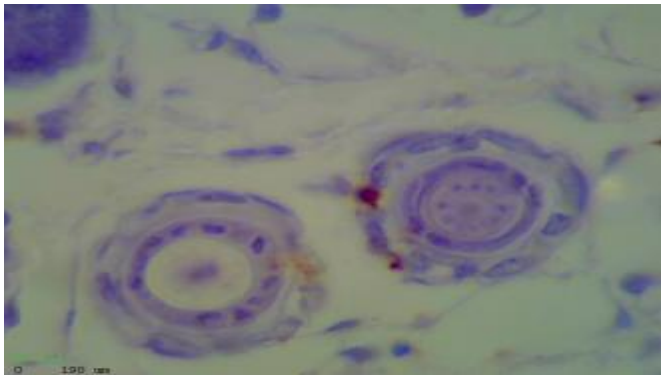

Fig 8: Day 14 lased group showing stromal expression of IL-1β