Irradiation effect of 780-805nm diode laser on wound healing in mice

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ABSTRACT

Background: Wound healing is a complicated, interactive, integrative process involving cellular and chemotactic activity, the release of chemical mediators and associated vascular response which includes number of phases: inflammatory phase, proliferative phase and remodeling phase. Low level laser therapy can be more effective in the three overlapping phases of wound healing. Biostimulation appears to have an effect on the cellular level, by increasing cellular function and stimulating various cells. The aim of present study was to evaluate histologically the effect of 780-805 diode laser the intensity of inflammation and pattern of epithelization in mice model.

Material and methods: The experimental study was performed on ninety six white albino mice. An incision of 1.5cm length was done on the face side of each mouse. Then the animals were divided into two main groups; control group which didn't receive laser irradiation while the other group was the lased group which exposed to single dose of 360J/cm of 780-805nm diode laser. Animals were subdivided into four subgroups according to healing periods. Histological specimens were taken at 1st, 3rd, 7th and 14th day for microscopical examination concerning inflammatory cells infiltration and epithelial cell layer thickness.

Results: Results show obvious reduction in inflammatory cell infiltration and more epithelization in laser treated wounds compared with control wounds. Statistical analysis showed a significant difference between two groups.

Conclusion: Low level diode laser (790-805nm) has beneficial effects in enhancement of soft tissue wound healing process histologically.

Keywords: low level laser therapy (LLLT), photobiomodulation. (J Bagh Coll Dentistry 2013; 25(Special Issue 1):48-52).

الخلاصة:

خلفية الموضوع: -تعتبر عملية شفاء الجروح عملية معقدة تتطلب فعالية خلوية وتجاذب كيمياوي وافراز وسائط كيمايوية واستجابة وعائية والذي يتضمن عدة مراحل:التهابية تكاثرية واعادة تشكيل. ويعتبر العلاج بالليزر الواطئ الطاقة فعالا اكثر في المراحل الثلاث الناتادخلة لعملية الشفاء. ويظهر تاثير الاستحاثة البايولوجية على المستوى الخلوي من خلال زيادة فعالية الخلايا وتحفيز انواع الخلايا. هدف الدراسة الحالية هو التقييم النسيجي لـاثير الليزر 780-805 نانوميتر على شدة الالتهاب وطريق تشكيل الطبقة الطلائية في جروح الفار .

المواد والطرائق: استخدم 96 فأرا ابيض تعرضوا جميعهم لعملية جرح قطعي في ناحية الوجه بطول 1.5سم . ثم قسمت الحيوانات الى مجموعتين رئيسيتين الليزر والقياس . تعرضت مجموعة الليزر لجرعة واحدة من اشعاع الليزر بعد عملية الجرح مباشرة بجرعة 360 جول\سم^2 .بعد ذلك تم اخذ العينات من كل مجموعة في اليوم الاول والثالث والسابع والرابع عشر من ايام الشفاء.

النتائج: بينت النتائج نقصا واضحا في انتشار الخلايا الالتهابية وزيادة سمك الطبقة الطلائية في مجموعة الليزر مقارنة بمجموعة القياس والنتائج الاحصائية اظهرت فرقا واضحا بين المجوعتين

الاستنتاجات: ان استعمال الليزر الواطئ الطاقة780-805نانوميتر له تأثير واضح على تسريع عملية شفاء الجروح نسيجيا

INTRODUCTION

Laser energy is a sort of electromagnetic energy which, depending on its source, can be converted into luminous energy, visible or not. Laser arrays are, therefore, a highly concentrated noninvasive kind of non-ionizing radiation that, in contact with different tissues, promotes thermic, photochemical, and nonlinear effects(1) .Several studies have indicated that laser arrays at low frequencies (low-level laser therapy, LLLT) are quite helpful in modulating different biological activities, such as trophic regenerative(2) anti-inflammatory(3) and analgesic effects(4) It has been suggested that biological properties of visible spectra of LLLT are probably a result of cellular photoacceptors and signaling pathway stimulation by light.

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Therefore, the absorption of monochromatic visible radiation by components of the cellular respiratory chain accelerates the transfer of electrons from NADH and FADH2 (produced in the Krebs cycle) to oxygen molecules to form (with the aid of protons) water molecules, harnessing the energy released by this transfer to the pumping of protons (H+) from the matrix to the intermembrane space. This gradient of protons formed across the inner membrane by this process of active transport forms a miniature battery.

Consequently, this mechanism increases the mitochondrial ATP production and protein synthesis. Furthermore, it was proposed that cytochrome c oxidase is the primary photoacceptor for both the red and infra-red range in mammalian cells (1) .Wound healing is a biological response to tissue injury. This highly controlled repair process is characterized by the movement of specialized cells into the wound site, in order to provide key signaling events required for the influx of connective tissue cells and a new

blood supply[5,6] Despite the fact that wound healing represents a reparative response to tissue insult, a long-term or over- induced inflammatory reaction is extensively implicated in promoting atypical patterns of healing, such as fibrosis, strictures, adhesions, and contractures [7]. Previous reports have demonstrated that LLLT is efficient in stimulating cellular proliferation and collagen synthesis in biological assays [8]. Variations in the wavelength and dose of phototherapy, as well as optical characteristics inherent to every single tissue, have been pointed out as relevant factors to assure the success of the laser-induced biological modulation. Moreover, the latter variable has been currently considered particularly important to evaluate the extent of the interaction between laser irradiation and cells [10].

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 didn't 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.

Method of assessment:

1. Evaluation of inflammatory cells infiltration:-It was made by examining many sections of each specimen by measuring the number of inflammatory cells per square of grid at power field (x40) magnification. The inflammatory cells that were investigated are (neutrophil, lymphocyte, macrophage and eosinophil).
2. Evaluation of epithelial cell layers thickness:- It was done by examining the surface of wound at the incision site of each specimen using computerized microscope by measuring ruler within the software of light microscope (Micros, Astruia). The measurement of epithelial thickness was performed by measuring the distance from outermost keratin layer to innermost basal layer of epidermis. In the current study, the descriptive and inferential statistics (independent t- test and analysis of variance (ANOVA) two way tests) were used in order to assess and analyze the results. Statistical probability (p) <0.05, 0.001.

RESULTS

As showed in [Table 1], the intensity of the inflammatory response was severe in absolutely all the cases of in control group in day 1 and 3. Besides, the leukocyte infiltrate was predominantly composed of neutrophils and lymphocytes, characterizing an acute inflammatory reaction. In general, neutrophils were distributed along the wound surface, particularly within the fibrinous exudate membrane [Figure 1 and 3] whereas lymphocytes were observed in the deeper regions of the specimens. Macrophages and eosinophils were observed in little number in day 1 and increased till day 7 to incline gradually till day 14. The wounds of the irradiated animals in day 1 presented a severe inflammatory response. Neutrophils and lymphocytes were also the most frequently observed leukocytic cells, but they appeared to be less abundant in this group in day 3, particularly neutrophils [Figure 2 and 4]. However, the pattern of distribution of these leukocytes throughout the wound was nearly similar to that seen in control group. In addition, the macrophages and eosinophils of laser group presented the very same profile found in control group. At the 7th day, the intensity of the inflammatory response in control group was predominantly mild, although it still presented macrophages. Besides, lymphocytes were the less abundant leukocyte and some blood vessels neoformation were observed spread out in the connective tissue, but this vascular component was less obvious as in laser group [Figure 5].The inflammatory response subsided in laser group; [Table 1]. Moreover, a remarkable neoformation of capillary blood vessels, mainly in the deeper regions of the healing area, was also observed [Figure 6]. Even though the presence of neutrophils could still be seen in some cases, their presence was not remarkable in any case. As indicated in the [Table 1], epithelization was seen to be highest level at the 7th day of surgery in both control and laser group [Figure 5 and 6] was seen in all the specimens but the laser group showed thicker epithelial layer than control group . On the 14 th day, epithelial neoformation was less and irregular in control group of the wound surface [Figure 7]. On the other hand, significant neoformation of the recovering epithelial tissue was observed in almost all the animals of laser group more than control group, but it was seen to be complete remodeled than that seen in day 7[Figure 8].

Table 1: Comparison between control and laser group (independent t-test)

Day	Parameter	Control		Laser		t-test	P-value	Sig.
		M	SD	M	SD			
1	Neutrophil	33.33	23.02	35.5	32.27	-.19	.85	NS
	Lymphocyte	8	10.92	8.58	9.93	-.14	.88	NS
	Macrophage	.58	1	2.75	3.76	-1.93	.666	NS
	Eosinophil	0.5	.522	1.5	2.46	-1.37	.18	NS
	Epithelization	69.34	25.87	102.95	38.28	-2.52	.01	S
3	Neutrophil	35.5	32.27	10.58	9.49	3.84	.002	HS
	Lymphocyte	16.92	18.4	8	8.27	1.53	.154	NS
	Macrophage	5.92	5.19	2.75	3.77	1.82	.08	NS
	Eosinophil	.33	.49	.75	.75	-1.6	0.123	NS
	Epithelization	86.31	38.63	138.5	49.48	-2.88	.008	HS
7	Neutrophil	15.92	20.95	11.92	10.97	.59	.56	NS
	Lymphocyte	17.16	21.23	6.17	2.97	1.75	.08	NS
	Macrophage	20.75	20.85	4.25	3.27	2.71	0.01	S
	Eosinophil	.17	.38	.33	.49	-.92	0.36	NS
	Epithelization	112.65	51.92	207.04	175.15	-1.79	0.08	NS
14	Neutrophil	7.66	12.11	8.75	6.04	-.28	0.952	NS
	Lymphocyte	10.66	10.21	5.00	2.92	1.68	.107	NS
	Macrophage	3.33	2.57	1.41	.9	2.44	.023	S
	Eosinophil	1	1.7	.41	.51	1.13	.27	NS
	Epithelization	112.07	64.19	98.22	.75	.6	.55	NS

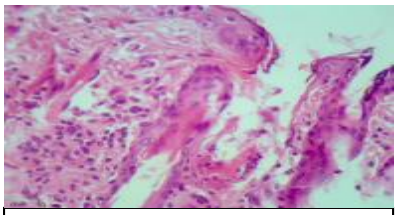

Fig.1: Control group/day1

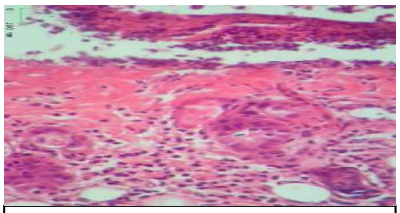

Fig.2: Lased group/day1

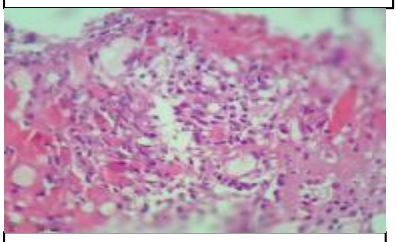

Fig.3: Control group/day3

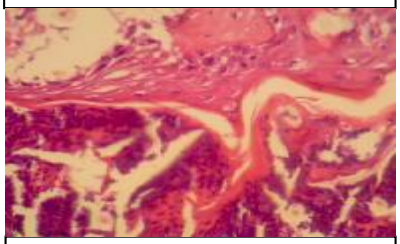

Fig.4: Lased group/day3

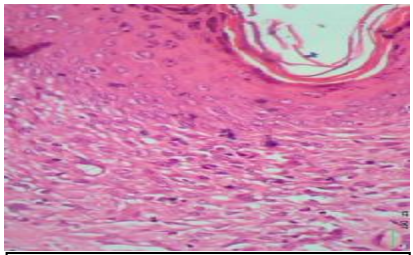

Fig.5: Control group/day7

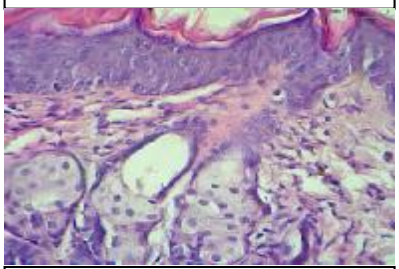

Fig.6: Lased group/day7

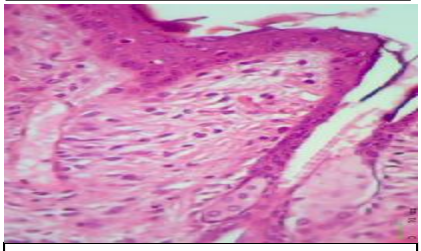

Fig.7: Control group/day14

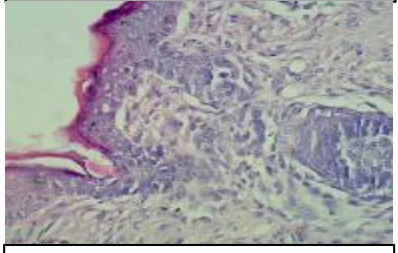

Fig.8: Lased group/day14

DISCUSSION

The induction of repair in wounds represents a dynamic process that involves the integrated actions of many cell types, extracellular matrix and chemical mediators. The inflammatory reaction represents the earliest event to take place after tissue injury, whose main function is to eliminate eventual microorganisms and provide wound cleaning. Subsequently, biological events, such as formation of new capillary blood vessels associated with progressive deposition and remodeling of collagen fibers will end in a complete repair of the injured area [6]. Inflammatory response is absolutely required to provide wound healing, but its long-term persistence has been considered one of the most important reasons of delay in the healing process [11]. Despite recent advances in improving the wound-healing process, many studies have still been performed looking at new strategies to stimulate the biological events that comprise the repair phenomenon [12]. Studies have demonstrated that laser arrays present some biostimulatory properties apparently able to accelerate wound healing of soft tissue injuries [13, 14] . Wavelengths in the 600-700 nm range have been employed in treating superficial tissues, such as cutaneous or mucosal wounds, whereas wavelengths between 780 and 950 nm have been applied in deep seated tissue insults, like bone fracture healing [15]. However, both positive and negative results have been reported in the literature, probably as a result of different protocols of photobiomodulation employed in as in vitro and in vivo biological assays.

The beneficial effects of adequate protocols of photobiomodulation on wound healing can be explained by considering its ability to stimulate several biological mechanisms responsible for triggering many phases of the repair of soft tissue injuries, including the induction of cytokines and expression of growth factors by keratinocytes and stromal cells [1]. On the other hand, negative responses can be a result of an unsuitable interaction between laser light and tissue components of the wound-healing process due to the application of inappropriate protocols of photoirradiation [10]. The dosage of energy applied in the photobiomodulation procedure is currently considered as an extremely relevant parameter to provide a significant improvement in repair [10]. A specific protocol of laser irradiation at 790-805nm has been previously reported which showed to be truly effective in improving the wound-healing process, possibly by promoting photostimulation of a variety of cell subsets, such as fibroblasts, myofibroblasts, and epithelial cells, at the same time modulating the inflammatory response [14]. In this study, we investigated the effectiveness of a increased energy dosage of laser irradiation in stimulating wound healing. We made use of the very same general protocol employed in a previous work [14]. In order to afford subsequent reliable comparisons between our findings and those recently reported. Within the protocol of photobiomodulation applied in this study, LLLT was able to induce a certain decrease in the intensity of the inflammatory reaction at both 1st and 3ed days after surgical procedures. In fact, a remarkable anti- inflammatory activity of LLLT has been reported within other different protocols [16,17]. The findings suggest that, this protocol of LLLT had shown ability to modulate the inflammatory response; it asserted that its effect might truly favor the acceleration of the healing process. However, this protocol of LLLT appeared to be successful in influencing the immunoinflammatory response, i.e., once it reduced the amount of neutrophils at the earlier stages of the wound-healing process irradiated wounds. This modulatory effect of LLLT over the acute inflammatory response might be a result of an important inhibitory role played by laser arrays, on the synthesis of prostaglandin, a chemical mediator widely supposed to provide chemotactic signals for polymorphonuclear neutrophils [18]. Therefore, LLLT might provide a short term acute inflammatory response in earlier stages of wound healing, which certainly would favor the process of wound repair. Epithelization is the process where epithelial cells on the edges of the wound or in residual skin appendages lose contact inhibition and migrate into the wound area. Simultaneously, additional epithelial cells are provided by the proliferation of immature keratinocytes in the basal layer .As keratinocytes are supposed to be a source of a variety of cytokines involved in remodeling the collagen fibers deposited at the final stages of repair, the development of epithelial lining is considered a relevant step of wound healing. Furthermore, the epithelial bridge is also responsible for removal of scab by dissolution of its attachments to the underlying connective tissue [6]. In this study, the process of wound surface epithelization was enhanced by LLLT in both stages of wound healing, although the advances in epithelial neoformation had been less apparent in 8 than in 14 days. These findings are supported by previous studies asserting that LLLT was shown to be effective in stimulating the migration of keratinocyte along the healing wound surface, probably as a result of the release of growth factors such as EGF (epidermal growth factor)

and TGF-á (transforming growth factor alpha) by irradiated macrophages [1,18,19]. Furthermore, LLLT was recently proved to act directly on keratinocyte-promoting epithelial cell proliferation in vitro [19]. This may be the reason for increased epithelization to be more intense in final than in early stages of the healing process .Once the wound is "cleaned," the inflammatory phase of the wound repair is gradually substituted by the proliferating phase as the healing process takes place. The latter is characterized by migration of fibroblasts into the wound area and consequent deposition of the collagen fibers required for the repair of tissue injury [11,6]. The results obtained in this study by using a 790-805nm of dose of 360J/cm^2 can assert that this protocol of photobiomodulation was successful in improving certain steps of wound healing, such as inflammatory profile and epithelization.

As conclusions; the LLLT protocol tested in this study improved wound healing in vivo. Nevertheless, further investigations are necessary to provide data about possible extrapolations of its effectiveness in wound repair in human beings.

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