International Journal of Health Sciences and Research
www.ijhsr.org ISSN: 2249-9571

Original Research Article

Photo Stimulatory and Photo Inhibitory Effect of Low Level Laser Therapy in Diabetic Wound Healing Dynamics- Preclinical Study

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Received: 25/03//2012 *Revised: 14/04/2012* *Accepted: 23/04/2012*

ABSTRACT

Background: The significances of laser photo stimulation are now accepted generally but the laser light facilitates wound healing and tissue repair remains poorly understood. So we have examined the hypothesis that the laser photo stimulation can enhances the collagen production and histological changes in diabetic wounds using excision wound model in Wister rat. Method: The circular wounds were created on the dorsum of the back of the animals. The animals were divided into two groups. The study group (N=12) wound was treated with laser with wave length of 660 nm at a dose of 7 j/cm2 and 15j/cm2 for five days a week until the wounds healed completely (wound closed on average 12th day in 7J/cm2 and 19th day in 15 j/cm2). The control group was sham irradiated (Wound closed on average of 28th day). Result: Significant increase in the hydroxyproline content and histopathological changes was observed in study group of 7 J/cm2 as compared to 15 j/cm2. The healing actions seem to be due to increased collagen deposition as well as better alignment and maturation. In conclusion the biochemical and histopathological findings confirms that laser photo stimulation facilitates the tissue repair process by accelerating collagen production in diabetic wound healing.
Key words: Laser photo stimulation; Wound healing, Hydroxyproline, histopathology, excision wound

INTRODUCTION

Chronic wound healing is an enigmatic and debilitating complication and poses a serious challenge in clinical practice. Wound healing and tissue repair are complex processes that involve a dynamic series of events including clotting,

inflammation, granulation tissue formation, epithelization, collagen synthesis and tissue remodeling. [1] The exact pathogenesis of the poor healing in diabetic wound healing is not clearly understood, but evidence from studies involving both human and animal models reveal several abnormalities in the various phases of wound healing process. [2-5] In recent years low intensity laser photo stimulation has gained considerable recognition and importance among treatment modalities for various medical problems including wound repair processes, musculo-skeletal complications, and pain control. [6-9] Clinical studies have shown low energy lasers to be effective as analgesics, and to accelerate the healing of injured tissue. [10-13] Although the beneficial effects of laser photo stimulation are now generally accepted, the mechanisms by which laser light facilitates diabetic wound healing and tissue repair remains poorly understood. Therefore, the objectives of the present study was to study the effect of different doses (7 & 15 J/cm2) of low level laser therapy on bio chemical properties and histopathological properties in diabetic wound healing as compared to control group

MATERIALS AND METHODS

C.1 Experimental animals, equipments (Laser Therapy, AutoCAD RL 14 Computer) [14]

C.2 Ethical committee clearance: The protocol of proposed work was submitted to the Manipal University ethical committee and the clearance was obtained for the experiment.

C.3 Animals: In-house bred albino Wister strain male rats were used in the study. The range of weight of animals was between 160 to 240 gm. All the animals were maintained in less than 12 hr day light environment. In each cage a maximum of three animals were housed. They were housed in polypropylene cages (29x22x14cm) with paddy husk bedding at 28c+1c. The animals were kept in a hygienic environment and the bed was changed on alternate days. All the animals were provided with water and food ad libitum. Breeding and maintenance of the animals were done as per the guidelines of Government of India for use of Laboratory animals (Government of India notifies the rules for breeding and conditioning animals experiments, proposed in the gazette of India Dec 15, 1998; which was reproduced in Ind. Journal of Pharmacology. 1999; 31:92-95) [15]

C.4 Experimental Design: The animals were anesthetized with intravenous ketamine of 2mg/kg body weight. The dorsal fur of the animals was shaved with electric clipper. The area of wound to be created was marked on the back of animals by methylene blue using circular stainless stencil. The full thickness 3 cm2 excisional wound was created along markings using toothed forceps, surgical blade (number 15) and pointed scissors. The area of the wound was recorded on transparency paper. All the wounds were kept open.

C.5. Induction of Diabetes in wistar Rats by Alloxan: The alloxan was injected intra peritonally to animals at 80 mg/kg body weight. And after seven days of stabilization period, blood samples were obtained from animals fasted over night and diabetes status was confirmed. Then ever week the blood sugar level was measured to confirm the diabetic status of the animals till completion of the study.

C.5 Laser Therapy Schedule: The low energy He-Ne laser provides wavelength of 660 nm by continuous mode. An average power of 7 J/cm2 and 15 J/cm2 (D= p x t /A) were provided through a fiber optic delivery system over the wound for 5 days a week until the wound healed completely in three different experiments.

C.6 Biochemical parameter: On the 5th, final day the granuloma tissue by 1cm wide by approximately 2 cm in length were collected from each animal for analysis. The wet weight of granuloma tissue was recorded. The tissue was dried in an oven at 60ºC and the dry weight noted again. Then hydroxyproline level was noted. Granulation tissue was observed and graded in I-IV subjectively depending upon the amount of granulation tissue present in both group.

C.7. Histopathological analysis: Sample collection and analysis: On day 4th day and on complete healing approximately 1cm tissue from wounded area and margin of approximately 0.5 cm of unwounded skin was taken for analysis.

DATA ANALYSIS

The biochemical parameter was analyzed using t test and histopathological was analyzed using Mann-Whitney U test.

RESULTS ANALYSIS

Table I, II, III,IV, V shows the effect of low intensity low level laser therapy irradiation with 7/Jcm^2 and 15 J/cm^2 on the hydroxyproline and histopathological changes concerned with the process of wound healing as compared to control group. A significant increase was observed in 7J/cm^2 of the wet granuloma tissue weight as well as in the dry weight as compared to 15 j/cm2. The comparison of the biochemical integrity of laser treated with 7j/cm^2 and control wounds showed that laser photo stimulation significantly increased biochemical stability of rats. In contrast, the control group has statistically insignificant values. The total collagen for laser treated wounds was significantly higher compared to control group.

Table: I. Wound healing effect of low intensity He Le laser treatment 7 J/cm^2 (Post wound day 5$^{th\ day}$ and final) as compared to control group

Day and parameter	Control group	Study group
5th Day		
Wet granuloma (mg)	220.3 ± 16.2	360.4 ± 25.1**
Dry granuloma (mg)	20.2 ± 3.5	78.2 ± 12.2**
Hydroxyproline	8.00 ± 0.81	29.50 ± 1.04***
12th Day (final)		
Wet granuloma (mg)	219.3 ± 15.1	391.4 ± 22.1**
Dry granuloma (mg)	22.2 ± 3.5	89.2 ± 14.2**
Hydroxyproline	17.50 ± 0.28	63.70 ± 0.70***

Values are mean ± S.D, n= 12; **p<0.001, ***p< 0.0001 vs control, Student's t-test.

Table II Granulation Tissue Grades Study versus Control Group (7 J/cm^2)

GRADE *	STUDY GROUP	CONTROL GROUP
Grade I	0	5
Grade II	1	7
Grade III	4	0
Grade IV	7	0

* The grades were depending on the subjective assessment of granulation tissues in both groups (from no granulation tissue to maximum granulation tissue).

Table III: Histopathological Analysis of Diabetic Wound (7 J/cm^2) as compared to control group (epithelisation)

N	Study		Control		Z	p
	Mean rank	Sum of rank	Mean rank	Sum of Rank		
12	32.79	787	19.21	399	-4.35	0.000

Table: IV. Wound healing effect of low intensity He Le laser treatment 15 J/cm^2 (Post wound day 5$^{th\ day}$ and final) as compared to control group

Day and parameter	Control group	Study group
5th Day		
Wet granuloma (mg)	162.3 ± 16.2	228.4 ± 25.1
Dry granuloma (mg)	10.2 ± 3.5	28.2 ± 12.2
Hydroxyproline	7.00 ± 0.81	9.50 ± 1.04
19th Day (final)		
Wet granuloma (mg)	172.3 ± 15.1	218.4 ± 22.1
Dry granuloma (mg)	11.2 ± 3.5	29.2 ± 14.2
Hydroxyproline	9.50 ± 0.28	10.70 ± 0.70

Values are mean ± S.D, n= 12; Student's t-test.

Table V: Histopathological Analysis of Diabetic Wound (15 J/cm^2) as compared to control group (epithelisation)

N	Study		Control		Z	p
	Mean rank	Sum of rank	Mean rank	Sum of Rank		
12	18.79	389	17.21	369	-4.35	0.306 (NS)

NS= not significant

DISCUSSION

The normal healing involves an initial inflammatory phase followed by fibroblast proliferation, formation of collagen fibres, shrinking and drying of scar. These phases are concurrent but independent of each other. The present study showed that the low intensity laser irradiation is reported to have anti-inflammatory activity, and proliferate action. This is demonstrated by increased hydroxyproline content, which is a reflection of increased collagen levels, which indicates better maturation and proliferation of collagen by increased collagen orientation and the finding were significantly more in laser treated with 7 J/cm2 as compared to laser dose of 15 J/cm2 and control group(Table I & IV).

This study shows that laser dose of 7J/cm2 low energy laser photo stimulation facilitates the wound repair process as evidenced by biochemical analysis and experimental observation of wound healing in rats. The experimental observation and biochemical findings of 7 J/cm^2 indicate that significant improvements occurred in the healing process following treatment. (Table I & II)

The normal healing involves an initial inflammatory phase followed by fibroblast proliferation, formation of collagen fibers, shrinking and drying of scar. These phases are concurrent but independent of each other. The present study showed that the low intensity laser irradiation is reported to have anti-inflammatory activity, and proliferate action. This is demonstrated by increased hydroxyproline content, which is a reflection of increased collagen levels, which indicates better maturation and proliferation of collagen by increased collagen orientation as evidenced by histopathological changes. Low energy laser photo stimulation began in the 1970s and has continued to gain widespread acceptance within the physical therapy profession for the treatment of a variety of medical conditions including impaired microcirculation and wound healing. [16]

The present study shows that low energy laser photo stimulation facilitates the wound repair process as evidenced by the biochemical analysis and histopathological observation of diabetic wound healing in rats. The influence of laser treatment on healing process was most pronounced in the biochemical findings and histopathological finding of 7/Jcm2 as compared to 15 J/cm2 and control group (Table II & V). The results of the laser dose of 7 J/cm^2 show that the production of collagen in diabetic wounds can be modulated by laser treatment. The content of the total collagen was significantly increased in laser treated wounds as compared to control group (Table I). The mechanism by which laser photo stimulation facilitates collagen production in wound healing was not clear with previous study. This effect may involve a variety of photo stimulating mechanisms. It is mainly due the fact that the laser energy at certain frequencies can modulate cell proliferation and release the growth factors from fibroblasts which facilitates the collagen synthesis and maturation. [17]

In the present study, based on the result analysis of the two different doses of laser it confirmed that 07 j/cm2 is a better photo stimulatory dose. We found more collagen production on 5th day in study group as compared to control group which indicates that collagen production can be further stimulated by laser irradiation. In the present study, we found that increased fibroblast proliferation (histopathological analysis on 4th day) is associated with increased collagen production (biochemical analysis on 5th day) and it is significantly increased in dose 07 j/cm2 as compared to 15 j/cm2 and control group. The

biochemical and histopahological analysis of 15 j/cm2 showed statistical insignificance as compared to control group.(Table I-V). According to (Mester et al 1971), [18] the primary contraction of the open wound can be stimulated by certain dose or inhibited by higher doses and no acceleration of the early contraction in wounds was observed with lower doses. Therefore, in the present study we found photo stimulatory effect with 07J/cm2 dose and photo inhibitory effect with dose 15 j/cm2.

CONCLUSION

In an effort to provide a more clinically relevant low energy laser dose on diabetic wound healing model, we investigated the effect of laser photo stimulation and photo inhibition dose in diabetic wounds. The biochemical and histopatholigical findings of especially of 7/cm2 dose from this study clearly indicate that low energy photo stimulation dose is beneficial in promoting wound healing. However, 15 j/cm2 is delayed the wound healing process and it indicates that higher dose leads to photo inhibitory effect.

ACKNOWLEDGEMENT

We acknowledge, Indian Council of Medical Research (ICMR), Government of India for approving a research grant to conduct "Photo stimulatory and Photo inhibitory effect of low level laser therapy in diabetic wound healing dynamics- A preclinical study: 5/3/8/41/RHN/2009

REFERENCES

1. Reddy GK. Laser photo stimulation accelerates wound healing in diabetic rats, Wound repair regeneration 2001; 9: 248-255.
2. Singer AJ, Clark RA. Cutaneous wound healing. N Engl J Med 1999; 341:738- 746.
3. Prakash A Pandit PN, Sharman LK. Studies in wound healing in experimental Diabetes. Int Surg 1974; 59:25-20
4. Goodson WH, Hunt TK. Studies of wound healing in experimental diabetes mellitus. J Surg Res 1977; 22:221-27.
5. Goodson WH, Hunt TK. Wound healing and the diabetic patient. Surg gynecol Obstet 1979; 149: 600-608.
6. Fahey TJ, sadaty A, Jones WG, Barber A, Smoller B, Shires GT. Diabetes impairs the late inflammatory response to wound healing. J Surg Res 1991; 50:308-313
7. Mester E, Mester AF, Mester A. The biomedical effects of laser application. Lasers Surg Med 1985; 5:31-39.
8. Yaakobi T, Maltz L, Oron U. Promotion of bone repair in the cortical bone of the tibia in rats by low energy laser (He-Ne) irradiation. Calcif Tissue Int 996;59:297-300.
9. Schindl A, Schindl M, Schon H, Knobler R, Bavelec, Schindl L. Low intensity laser irradiation improves skin circulation in patients with diabetic Microangiopathy. Diabetes Care 1998; 21:580-584.
10. Schindl A, Schindl M, Pernerstorfer-Schon H, Schindl L, Low intensity laser therapy: a review. J Invest Med 2000; 48:312-326.
11. Abergel RP, Meeker CA, Lam Ts, Dwyer RM, Lesavoy MA, Uitto J. Control ofconnective tissue metabolism by lasers: recent developments and future Prospects. J Am Acad Dermatol 1984; 11:114201150.

12. Lam TS, Abergel, RP, Castel JC, Dwyer RM, Lesavoy MA, Uitto J. Laser stimulation of collagen synthesis in human skin fibroblast cultures. Lasers LifeSci 1986; 1:61-77.
13. Lyons RF, Abergel RP, White RA, Dwyer RM, Castel JC, Uitto J.Biostimulation of wound healing in vivo by a He-Ne laser. Ann Plast Surg 1987; 18:47-50.
14. Maiya AG, Kumar P, Nayak S. Wound area measurement in experimental animals: Conventional graph paper method versus AutoCAD RL 14 Computer analysis. Physiotherapy 2002; 1 21-24
15. Government of India notification: Rules for breeding and conducting animal experiments. Indian journal of pharmacology 1999; 31: 92
16. Kameya T, Ide S, Acorda JA, Yamada H, Taguchi K, Abe N. Effect of different wavelength of low-level laser therapy on wound heali8ng in mice. Laser Therapy 1995; 7:33-36.
17. Steinlechner C, Cyson M. The effects of low level laser therapy on the proliferation of keratinocytes. Laser Ther 1993; 5:65-73.
18. Mester E, Spiry T, Szende B, Tota JG (1971). Effect of laser rays on wound healing. Am J Surg; 122:532-535.

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