

Original Article

Effect of Low Level Laser Therapy on the Surgical Wound Healing in Hamster

Neda Nasirian , Ali Nasirian

Dept. of Pathology, Qazvin University of Medical Sciences, Qazvin, Iran

ABSTRACT

Background and Objectives: Accelerating wound healing is a very important factor for patients to return to ordinary life. Laser seems to have positive effect on cutaneous wound healing. This study aimed to investigate the effect of red light laser 630 nm on cutaneous surgical wound in hamster and to compare outcomes such as angiogenesis, number of fibroblasts and collagen formation in intervention and control group.

Materials and Methods: This experimental study carried out with collaboration of Qazvin University of Medical Sciences with Mehregan Pet Clinic in winter of 2009. Thirty suri hamsters were randomly allocated in two groups and four parallel wounds were made on their backs. The intervention group was radiated with red-light laser 630 nm. After 2,5,10 and 14 days, skin biopsy were obtained and number of blood vessels, fibroblast and collagen production were compared with the control group. Data were analyzed using independent sample student t test using SPSS version 10 and *P* value less than 0.05 was considered significant.

Result: Evaluation of wounds in experimental and control groups showed significantly increased number of vessels and fibroblasts as well as collagen production in laser radiating group (*P*<0.05).

Conclusion: Laser therapy (630 nm) can accelerate wound healing in comparison with control group.

Keywords: Laser, Wound Healings, Hamster

Received: 14 December 2011

Accepted: 21 May 2012

Address communications to: Dr Neda Nasirian, Department of Pathology, Qazvin University of Medical Sciences, Qazvin, Iran.

Email: drsnasirianqums@gmail.com

Introduction

Acceleration of cutaneous wound healing has always been a very important factor after surgery for rapid recovery and returning to ordinary life style. Wound healing process included multiple stages such as infiltration of inflammatory cells, migration and proliferation of fibroblasts, synthesis of Extracellular Matrix proteins (ECM) such as collagen, connective tissue and parenchymal remodeling and then collagenization in order to increase wound strength (1). After day 3, granulation tissue progressively invades the incision space and collagen fibers start to present. By day 5, neovascularization is maximal and fibroblasts proliferate. During the second week, accumulation of collagen and proliferation of fibroblasts continues (2).

Low Level Laser Therapy (LLLT) is a form of phototherapy that involves the application of low power monochromatic and coherent light to lesions to stimulate wound healing. It can increase the speed, quality and tensile strength of tissue repair (5).

The effect of LLLT are photochemical, not thermal and the response of cells due to changes in photo acceptor molecules (known as chromophores) (6).

The exact mechanism of action of LLLT has not been completely understood, but it can stimulate metabolism (7). The effect of LLLT including wound epithelialization, reduction of edema and inflammation, increase in granulation tissue, fibroblastic proliferation, extracellular matrix synthesis and neovascularization, all of which lead to better tissue oxygenation and nutrition and enhance wound healing (8-10). Previous studies were performed which show the effect of laser on acceleration of wound healing in animals such as rat (11,12) also in human wounds such as diabetic foot and other ulcers (13).

This researches show the effect of laser therapy with different wavelengths on acceleration

of wound healing. In this study, we assessed the effect of red-light laser (630nm) in wound healing in hamster and compared it with control group.

Material and Methods

This experimental study was carried out in small animal clinic of Mehregan and Qazvin University. All animal procedures were in accordance with the declaration of Helsinki and the guide for the care and use of laboratory animals.

We performed our study on total 30-suri hamsters (random male and female). They were randomly divided into two groups including 15 experimental and 15 controls. After anesthesia, we performed four parallel surgical scraps on the back of hamster measuring each one 1cm in both groups. Red light laser 630nm was radiated 6 times, each time 10 seconds to 15 hamster of experimental group.

Then, biopsies were taken in day 2, 5, 10, and 14 after radiation from both groups. Samples were sent in formalin 10% to the laboratory. Tissue processing and sectioning of paraffin-embedded tissue were done and then slides were stained with hematoxylin and eosin (H&E).

Slides were studied by the pathologist. Number of vessels in field 40 was counted in day second and day fifth samples in both groups. Median number of fibroblasts in field 100 was counted in day fifth and day tenth samples in both groups. For day fourteenth specimen samples, trichrome staining was performed and qualitative estimation for collagen formation in two groups was done. Data were analyzed using independent sample student *t* test using SPSS version 10 and *P* value less than 0.05 was considered significant.

Results

Mean number of vessels on the second day in control group was 19.47 ± 2.03 and in 21.4 ± 2.06 in intervention group ($P = 0.015$) (Table 1).

Table 1- Number of vessels in control and laser treated group in samples taken on second day

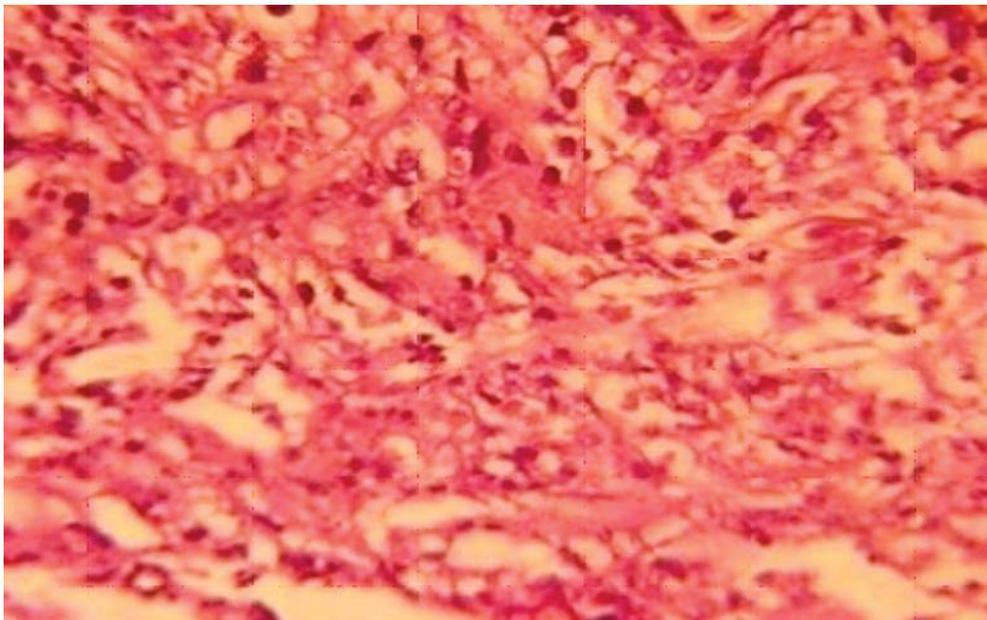
	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15
Control	18	18	20	18	19	20	19	21	18	22	19	17	20	13	25
Laser radiated	21	24	25	19	21	21	21	21	20	22	22	18	19	22	25

These numbers were 32 ± 2.2 and 34.67 ± 3.15 respectively, on the fifth day ($P=0.012$).

Table 2- Number of vessels in control and laser treated group in samples taken on fifth day

	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15
Control	35	32	30	35	31	29	33	31	30	34	32	30	29	35	34
Laser radiated	38	35	35	38	35	30	35	35	30	40	33	32	30	36	38

On this day, the mean and standard deviation of number of fibroblasts were 65.87 ± 3.11 and 61.27 ± 1.67 , respectively ($P<0.001$).

**Fig. 1 a & b** - Comparison of vessels number in fifth day in control and laser treated group**Table 3-** Number of fibroblasts in control and laser treated group in samples taken on fifth day

	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15
Control	60	65	60	60	61	63	61	59	62	60	64	61	60	61	62
Laser radiated	65	70	65	65	65	70	65	60	70	65	65	63	62	68	70

On 10th day, these numbers were 53.37 ± 1.64 and 10.13 ± 3.18 , respectively ($P<0.001$).

Table 4- Number of fibroblasts in control and laser treated group in samples taken on tenth day

	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15
Control	55	60	60	60	60	53	59	61	56	59	58	59	60	58	60
Laser radiated	70	70	70	70	70	65	70	70	80	70	70	70	63	72	67

Qualitative collagen production with trichrome staining on fourteenth day samples show significantly increased collagen production in experimental groups in comparison to intervention group.

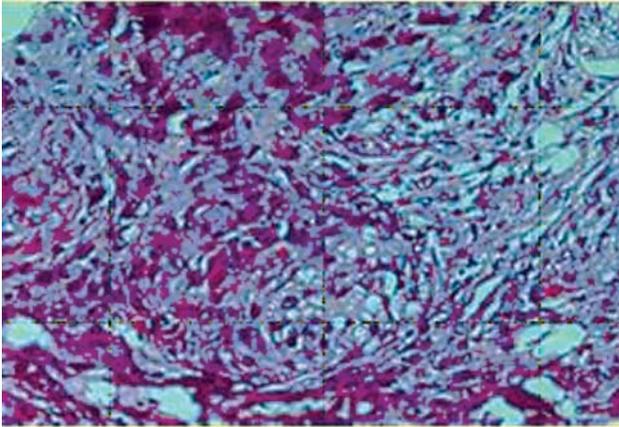


Fig. 2- Increased collagen production in day fifteenth specimens in laser treated group (Trichrome staining)

Discussion

Histological evaluation of wounds in two groups and the statistical analysis of data revealed significant difference between control and laser treated hamsters in mean number of vessels and number of fibroblasts. Studies have shown that laser therapy in wounds can increase cellular content and enhance granulation tissue formation, collagen deposition, and fibroblasts production (11, 12). The amount of total collagen was significantly increased in laser treated wounds over control group; also, it accelerates the production of collagen (13).

Multiple basic biological mechanisms can explain the effect of LLLT on wound healing including the induction of production of cytokines and growth factors, which are responsible for the many phases of wound healing. There is

a report that HeNe laser (632.8 nm) increased both protein and mRNA levels of IL-1 α and IL-8 in keratinocytes, which are responsible for the initial inflammatory phase of wound healing. LLLT can also induce production of cytokines, which are responsible proliferation, and migration of fibroblast, such as bFGF, HGF and SCF can increase growth factors such as VEGF, which is responsible for the neovascularization. TGF- β , which is a growth factor responsible for collagen synthesis induction from fibroblasts, upregulates by LLLT. Wavelengths in the 600-700 nm range are recommended for treating superficial tissue, and wavelengths between 780 and 950 nm for deeper-seated tissues, because of longer optical penetration distances through tissue. Helium Neon (632.8 nm) and laser diodes between (633-670 nm) are absorbed by the mitochondriacytochroms (mainly cytochrom C oxidase) and known to act mainly on superficial epithelial tissue due to limited penetration of red light (14). Acceleration of surgical wound healing help patients recover to normal life.

In study, we evaluated the effect of low-level laser therapy (630nm) on acceleration of wound healing in hamster. Our study showed that laser therapy could significantly increase number of vessels in second and fifth days. Laser can stimulate proliferation of fibroblasts and increase its number (in day 5 and 10). It is significantly effective in collagen production and can promote process of collagenization (in day 14)

Conclusion

Our study suggests that low-level laser therapy (630nm) can accelerate and promote surgical wound healing in hamsters.

Acknowledgement

This research was funded by Qazvin University of Medical Sciences and was performed through collaboration with Mehregan Pet Clinic in Tehran. The authors declare that there is no conflict of interests.

References

1. Houreld N, Abrahamse H. Low-level laser therapy for diabetic wound healing. Source: The diabetic foot, Date: 12/22/2005, Available online AT: http://www.mylightwave.com/docs/HighBeamResearch_Low-level_laser_therapy_for_diabetic_foot_wound_healing._Wound_care_.pdf. Last accessed 22 Dec 2011.
2. Bolton P, Dyson M, Young S. The effect of polarized light on the release of growth factors from the U-937 macrophage – Like cell line. *Laser Ther* 1992;4(1):33-8.
3. Dyson M, Young S. Effect of Laser Therapy on Wound Contraction and Cellularity in Mice. *Lasers Med Sci* 1986;1(2):125-30.
4. Kumar V, Abbas AK, Fausto N. Robbins and Cotran Pathologic Basis of Diseases. 8th ed. Saunders:New York;2007.
5. Walsh LJ. The current status of low level laser therapy in dentistry. Part 1. Soft tissue applications. *Aust Dent J* 1997;42(4): 247-54.
6. Tunér J, Hode L. laser therapy: Clinical practice and scientific background. 1st Sweden:Prima books;2002.
7. Pinheiro AL, Carneiro NS, Vieira, AL, Brugnera A Jr, Zanin FA, Barros RA, *et al.* Effects of low level laser therapy on malignant cells in vitro study. *J Clin Laser Med Surg* 2002;20(1):23-6.
8. Ohshiro T, Calderhead RG. Progress in low-level laser therapy. John Wiley & Sons: England;1991.
9. Steinlechner CWB, Dyson M. The effects of low level laser therapy on the proliferation of keratinocytes. *Laser Ther* 1993;5:65-73.
10. Loeveschall H, Arenbolt-Bindslev D. Effect of low-level diode laser irradiation of human oral mucosa fibroblasts in vitro. *Laser Surg Med* 1994; 14(4):347-54.
11. Reddy GK, stehnoBittel L, Enwemeka CS. Laser photostimulation accelerates wound healing in diabetic rats. *Wound Repair Regen* 2001; 9(3):248-55.
12. YU W, Naim JO, lanzafame RJ. Effects of photostimulation on wound healing in diabetic mice. *Laser Surg Med* 1997;20(1):59-63.
13. Matic M, Lazetic B, Poljacki M, Duran V, Ivkov-Simic M. Low-level laser irradiation and its effect on repair processes in the skin. *Med Pregl* 2003; 56(3-4):137-41.
14. HamblinMR. Mechanism of low-level light therapy. Available online at www.mgh.harvard.edu/wellman/people/mhamblin.asp.