



COMPARISON OF RED AND INFRARED LASER EFFECT ON WOUND HEALING

Neda Nasirian

Assistant Professor Of Qazvin University Of Medical Science, Qazvin, Iran.

Ali Nasirian*

Vet Surgen, Mehregan Clinic, Tehran, Iran. *Corresponding Author

ABSTRACT Usefull effect of laser is known by many scientist and it is used for wound healing ,tendon or muscle repair andsome other disease.In this study ,we want to compair effect of red and infrared layser on wound healing. **MATERIAL AND METHOD:** We select 30 male rats and made skin wound on their back and divided them into four groups ,Control group which wounds dont recieved laser radiation ,second group with Red layser radiation and third group with Infra red layser radiation. Then we got skin specimens from all rats in daye 2 ,5 for vascular count in field 40 and and thinth day for conting of fibroblasts.Then we analysed results with SPSS system and compairs effects of each group. **RESULTS:** Analysis show mean of vesseles number in second group was significantly more in group of in frared radiation in camparition to red layser radiation in second day.But it wasnot significant in fifth days specimens. In tenth day specimens ,mean of fibroblastic proliferation was significantly more in group of infrared radiation in camparition to red layser radiation.In both groups ,laser induce significantly more number of vesseles in second and fifth days and more fibroblasts in tenth day specimens in comparision of control group. **CONCLUSION:** Infrared layser induce more vascular proliferation and more fibroblastic proliferation with radiation of infrared layer incompairition of red layser radiated group .

KEYWORDS : Repair, Fibroblas, Vascular Proliferation**INTRODUCTION**

Skin wound repair is a complex process which has multiple steps ,at first ,inflammatory cells and fibrin deposits souround ulcer ,(inflammatory phase) and then ,because of cytocynes ,produced from macrophages such as VEGF,vascular proliferation start and reach to peak in fifth day and form granulation tissue.Then cytocynes induce fibroblastic proliferation and then ,collagen production performs.(1)Photobiomodulation employs monochromatic light in the optical region of red and infrared lasers to treat various tissues in a non-destructive and non-thermal fashion Treatment is based on the ability of light to alter the cell metabolism, particularly as a result of being absorbed by mitochondria and cytochrome C oxidase [2]. laser therapy opened a newperspective in wound management because of its clinical efficacy in tissue healing.The basic principle of laser therapy is that monochromatic light has a wavelength that is capable of affecting cell behavior without significant heating effects Laser benefits, including stimulation or inhibition of physiological, biochemical, or proliferative activities, are associated with biomodulation using laser therapy.

Beneficial effects demand a threshold of radiation exposure, and the variation of the laser fluency is calculated according to the desired effect.(3) The effects are dose-dependent, and high energies, within specific limits for each tissue ,laser combination, may be harmful. Photobiomodulation employs monochromatic light in the optical region of red and infrared lasers to treat various tissues in a non-destructive and non-thermal fashion . Treatment is based on the ability of light to alter the cell metabolism, particularly as a result of being absorbed by mitochondria and cytochrome C oxidase . Low-level laser therapy (LLLT) applied has demonstrated positive and protective effects on muscle repair, including the modulation of the angiogenesis [5,6,7], inflammatory process [6-7],collagen remodeling [6,7,8,9] as well as the formation of immature muscle fibers .However, the combination of the both administration times together has not been clarified.Enwemeka et al. and Woodruff et al. with meta-analyzes have shown that red and infrared laser can collaborate in wound repair, they stimulate collagen formation, pain control, mast cell degranulation and angiogenesis within an interval greater than 8.25J/cm² and less than 130J/cm² , being better in the range between 19 and 24J/cm².(8)

Healthy persons rarely demand treatment for wound healing.The use of lightmay have favorable effects on wounds that do not heal. Delays in wound healing usually result from complications such as bad nutrition, diabetes,malignancy, ischemia due to vascular disease, and advanced age.(9) The systemic effect of LLLT also was evaluated in ulcers and gangrene of patients with diabetes in a study by Schindl et al who found significant improvement in local microcirculation.(10)The use of light may have favorable effects on wounds that do not heal.delays in woundhealing usually result from complications such as bad nutrition, diabetes,malignancy, ischemia due to vascular

disease, and advanced age(11) The combined application of red and infrared lasers resulted in the most evident systemic effect on the repair of skin wounds produced in rats.(12-13-14) It was also found that wound repair were higher when laser therapy was adjusted with higher power intensity and shorter wavelengths(15-16) . It was also found that wound repair were higher when laser therapy was adjusted with higher power intensity and shorter wavelengths⁽¹⁷⁾. The same authors demonstrated in different years, that the increase in application frequency from three to five weekly sessions did not result in changes in wound repair, but higher frequencies can inhibit the repair of these lesions which collaborates with this thesis articles with good results using therapy sessions on alternate days⁽¹⁸⁻¹⁹⁻²⁰⁾ .

MATERIAL AND METHOD.

This was an experimental study which was carried out in small animal clinic of Mehregan in Tehran and we sent specimens for pathologic evaluation in 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 a Total 25 suri hamster (random male and female). They were randomly divided them into three groups including 10 experimental for red laser irradiation ,10 for infrared irradiation and 10 as control groups . After anesthesia we perform four parallel surgical scrap on the back of hamster measuring each one 1cm in both groups . Red lightlaser nm was radiated 6 times, each time 10 seconds to 10 hamsters of each experimental groups. Red laser (aluminium gallium indium phosphide (AlGaInP); $\lambda = 685\text{nm}$; infrared laser (gallium-aluminum-arsenide (GaAlAs); $\lambda = 830\text{nm}$; Then biopsy were taken in days fifth and tenth after radiation from both experimental and control groups. Sample were sent in formalin 10% to laboratory. Tissue processing and sectioning of paraffin embedded tissue were done and then slides stained with H&E (hematoxylin and Eosin).We prepared pathology slides for specimens in fifth day for each group also specimens of tenth day.We count vascular number in slides of fifth day (granulation tissue phase also fibroblasts in specimens of tenth day (fibroblastic proliferation phase)for each groups and compare results of each group together.Then we use t test and SPSS for finding of being meaningful of dataes .

RESULTS

Second day samples show mean number of 19/4 of vesseles in control group in comparison to infra red lasertreated group with mean number of vesseles of 25/2. T test show significant difference between twogroups ($P < 0.015$) Also mean number of 19/4 of vesseles in control group in comparison to red lasertreated group with mean number of vesseles of 22/2. T test show significant difference between twogroups ($P < 0.015$).Between two groups of infrared laser and red layser irradiation,t test show meaningful difference ($P < 0.015$)which means more influence of infrared layser in comparithion of red layser in vascular proliftraion .[Table 1].

In fifth day samples also mean number of vesseles in 10 and hamster was 36 in infrared light radiated group and 34.67 in red laser radiated group which both show significant difference in comparison to control group. $p < .0001$ But difference between infra red and red laser treated groups was not meaningful ($p = .03$) (Table 2) Number of fibroblasts estimated in experimental (infra red laser treated) and control group (61/2 and 66/6) which show significant difference in kruskal test between two groups. ($P < 0.0001$) Also Number of fibroblasts estimated in experimental and control group (in red laser treated groups 61/2 and 65/87) which doesn't show significant difference in Kruskal test between two groups. $P = .06$ (Table 3)

Number of fibroblasts in tenth day specimens estimated in experimental (Infrared) and control group (58.87 and 71.73) which show significant difference in kruskal test between two groups. ($P < 0.0001$)

Also Number of fibroblasts estimated in experimental (Red) and control group (in infra red laser treated groups) which show significant difference in kruskal test between two groups. ($P < 0.0001$) (Table 4)

DISCUSSION

A Pinheiro et al. compared laser therapy (685nm) and polarized light (400 - 2000nm) in Wistar rats, radiating intercalary days for 7 days with fluency ranging from 20 or 40J/cm² and 40mW. Histological analysis demonstrated that the laser and polarized light with 20J/cm² induced increase in collagen fibers, but the myofibroblasts were increased only with polarized light. (21) Another study (Gál, P., Vidinsky, et al) evaluated the systemic effects of laser irradiation on healing of wounds produced on the skin of rats. Two parallel incisions were made on the back of each animal and immediately sutured. After surgery, one wound of each rat was irradiated with LLLT at wavelength 670nm and a daily fluency. The comparison with non irradiated wounds showed that laser stimulation accelerated the inflammatory phase, as well as the proliferative and maturation phases. (22) The systemic effect in repairing skin wounds has been reported with red laser (685nm AlGaInP with, 30mW, 20J to 667s), infrared laser (GaAlAs with 830nm, 50mW, 20J and 401s) and the association of both. Rodrigo et al. used 36 Wistar rats, with three dorsal wounds, where only the center wound was treated every other day. Histological analysis showed superiority of the combination of lasers, where also the best repair of wounds occurred far of focus of treatment, indicating systemic reparative effect of the laser. (10) study to define the ideal dose of laser light in the treatment of skin wounds on the backs of rats. (23) In the comparison of fluency of 10, 20, and 30J/cm² applied three times weekly, the best results were obtained with 20J/cm². Then, the best results for skin wounds were verified to helium-neon (HeNe; 632.8nm), gallium-aluminum-arsenide (GaAlAs; 780nm), argon (488-514nm), GaAlAs (830nm), helium-cadmium (442nm), and krypton (640-670nm). Their findings confirm that the depth of laser penetration, which is associated with the wavelength which used, is not proportional to the biomodulation effects of laser therapy. (24-25) Based on these outcomes reported by Al-Watban and Zhang, 2 other authors used a fluency of 20J/cm² to evaluate the healing of skin wounds on the back of rats. (4-5) Their analysis showed that wave lengths between of 685nm and 830nm (10J/cm² each; total=20J/cm²) promoted the best skin wound healing. (26) The comparison with non irradiated wounds showed that laser stimulation accelerated the inflammatory phase, as well as the proliferative and maturation phases. The regeneration of damaged epidermis and the repair of striated muscle were also positively stimulated. (27-28) From the 386 articles identified, only four studies were included, with two LLT used with single wavelength (1: 904 nm vs. control and 2: 940 nm vs. 808 nm vs. 658 nm vs. placebo) and two LLT used probe cluster. One study compared to different single wavelengths showed a significant 71% reduction of the PU and an improved healing rate in which 47% of PU healed completely after 1 month of therapy with the use of LLT with a wave length of 658 nm compared with other lengths. The other analyzed wavelengths were not significant in the assessed outcomes. Significant results were observed in the use of LLT with a 658 nm wavelength, and no evidence was found for use of wavelengths above that for the treatment of PU. Therefore, we also found no evidence in the laser used to probe the cluster. (29) It was also found that wound repair were higher when laser therapy was adjusted with higher power intensity and shorter wavelengths. (30) In our study, second day samples show mean number of 19/4 of vesseles in control group in comparison to infra red laser treated group with mean number of vesseles of 25/2. T test show significant difference between two groups

($P < .0001$) [Table 1]. Also mean number of 19/4 of vesseles in control group in comparison to red laser treated group with mean number of vesseles of 22/2. T test show significant difference between two groups ($P < .0001$). Between two groups of infrared laser and red laser irradiation, t test show meaningful difference ($P < .0001$) which means more influence of infrared laser in comparison of red laser in vascular proliferation.

In fifth day samples also mean number of vesseles in 10 and hamster was 36 in infrared light radiated group and 34.67 in red laser radiated group which both show significant difference in comparison to control group. $p < .0001$. Table (2)

After wound Number of fibroblasts estimated in experimental (infra red laser treated) and control group (61/2 and 66/6) show significant difference in kruskal test between two groups. ($P < 0.0001$) Also Number of fibroblasts estimated in experimental and control group (in red laser treated groups 61/2 and 65/87) which doesn't show significant difference in Kruskal test between two groups. $P = .06$. Table (3)

CONCLUSION

Our analysis data showed that both lasers (red and infrared) induce significant difference in number of vessels in second and fifth days also number of fibroblasts in day of five and ten of wound healing. But when we compare results of radiation of red and infrared laser together, infrared laser shows significantly more effects on vascular proliferation in second day also there are significant difference between these two groups in fibroblastic number in day ten after ulceration.

Also our result showed that there is no significant difference between effect of both laser (red and infra red) in vascular number in field 40 or fibroblasts number in field 40 in fifth day specimens of two groups.

Acknowledgment

We appreciate from pathology lab of Boali hospital of Qazvin and from MRS Bozorgy for preparing of specimens.

Ethical rules

We followed all Helsinki rules about animals and procedure was after skin anesthesia and did not kill rats.

Financial support

This study was confirmed by ethical committee of Qazvin university and financial support was performed by Qazvin university medical science. number of contract: 28/20/830

Conflict of interest

There is not any conflict.

Table 1: number of vesseles in control and laser treated group in day second days

1	2	3	4	5	6	7	8	9	10	Numbers
18	18	20	18	19	20	19	21	18	22	Control group
22	23	23	27	23	26	26	25	28	24	Infra red Laser radiation
21	24	25	19	21	21	21	20	22	22	Red laser radiation

Table 2: Number of vesseles in day fifth

1	2	3	4	5	6	7	8	9	10	Numbers
35	32	30	35	31	29	33	31	30	34	Control group
40	36	30	40	34	30	38	35	32	40	Infra red Laser radiation
38	35	35	38	35	30	32	33	30	36	Red laser radiation

Table 3: Number of fibroblasts in fifth day

1	2	3	4	5	6	7	8	9	10	Numbers
63	60	65	60	60	61	63	61	59	62	Control group
71	62	74	62	65	67	70	60	70	71	Infra red Laser radiation
65	65	70	65	65	70	65	60	70	65	Red laser radiation

Table 4: Number of fibroblasts in tenth day

1	2	3	4	5	6	7	8	9	10	Numbers
63	55	60	60	60	60	53	61	59	59	Control group
71	62	74	62	65	67	70	60	70	71	Infra red Laser radiation
65	70	70	70	65	70	65	70	70	70	Red laser radiation

REFERENCES

1. Vinay Kumar, AbolK. Abbas, Junk Aster, Robbins Basic pathology, publisher Tenth edition 2017, Chapter 3, page 110
2. Naira Figueiredo Deana1,2; Nilton Alves3,4; Vanderlei Salvador Bagnato5 & Paulo

- SandovalKaru TI Effects of Low-Level Laser on the Repair of Orthodontically Induced Inflammatory Root Resorption: A Systematic Review of Studies in Rats nt. J. Morphol.,37(3):977-984, 2019.
3. Ribeiro BG, Alves AN, Santos LA, Fernandes KPS, Cantero TM, Gomes MT, et al. The effect of low-level laser therapy (LLLT) applied prior to muscle injury. *Lasers Surg Med.* 2015. July 6 10.1002/lsm.22381
 4. Alexander cunha, Daniel hambertopoza, Jeao Feliz MoraesJoao Batista „Blessmann weber. Analysis of the Systemic Effect of Red and Infrared Laser Therapy on Wound Repair. *Photomedicine and laser surgery* • September 2009
 5. Ribeiro BG, Alves AN, Santos LA, Fernandes KPS, Cantero TM, Gomes MT, et al. The effect of low-level laser therapy (LLLT) applied prior to muscle injury. *Lasers Surg Med.* 2015. July 6 10.1002/lsm.22381
 6. Alves AN, Fernandes KPS, Deana AM, Bussadori SK, Mesquita-Ferrari RA. Effects of low-level laser therapy on skeletal muscle repair: a systematic review. *Am J Phys Med Rehabil.* 2014;93(12): 1073–1085. 10.1097/PHM.0000000000000158
 7. Alves AN, Fernandes KPS, Melo AV, Yamaguchi RY, França CM, Teixeira DF, et al. Modulating effect of low level-laser therapy on fibrosis in the repair process of the tibialis anterior muscle in rats. *Lasers Med Sci.* 2014;29(2): 813–821. 10.1007/s10103-013-1428-9
 8. Enwemeka CS, Parker JC, Dowdy DS, Harkness EE, Sanford LE, Woodruff LD. The efficacy of low-power laser in tissue repair and pain control: a meta-analysis study. *Photomed Laser Surg.* 2004;22(4)
 9. Rabelo, S.B., Villaverde, A.B., Nicolau, R., Salgado, M.C., Melo Mda, S., Pacheco, M.T. (2006). Comparison between wound healing in induced diabetic and nondiabetic rats after low-level laser therapy. *Photomed. Laser Surg.* 24, 474–479
 10. Schindl, A., Heinze, G., Schindl, M., Pernertorfer-Schön, H., Schindl, L. (2002). Systemic effects of low-intensity laser irradiation on skin microcirculation in patients with diabetic microangiopathy. *Microvasc. Res.* 64, 240–246.
 11. Rabelo, S.B., Villaverde, A.B., Nicolau, R., Salgado, M.C., Melo Mda, S., Pacheco, M.T. (2006). Comparison between wound healing in induced diabetic and nondiabetic rats after low-level laser therapy. *Photomed. Laser Surg.* 24, 474–479.
 12. Simone Marja Rodrigo 1, Alexandre Cunha et al. Analysis of the Systemic Effect of Red and Infrared Laser Therapy on Wound Repair, doi: 10.1089/pho.2008.2306
 13. Da Silva JP, da Silva MA, Almeida AP, Lombardi Junior I, Matos APLaser therapy in the tissue repair process: a literature review. *Photomed Laser Surg.* 2010 Feb;28(1):17-21. doi: 10.1089/pho.2008.2372.PMID: 19764898 Review.
 14. Laser photobiomodulation of wound healing: a review of experimental studies in mouse and rat animal models. Peplow PV, Chung TY, Baxter GD. *Photomed Laser Surg.* 2010 Ju
 15. Laser photobiomodulation of wound healing: a review of experimental studies in mouse and rat animal models. Peplow PV, Chung TY, Baxter GD. *Photomed Laser Surg.* 2010 Jun;28(3):291-325. doi: 10.1089/pho.2008.2446.PMID: 19995230 Review.
 16. Fernando José Camello de Lima, 1,* Fabiano Timbó Barbosa, 2 and Célio Fernando de Sousa-Rodrigues 1, Use alone or in Combination of Red and Infrared Laser in Skin Wounds, *J Lasers Med Sci.* 2014 Spring; 5(2): 51–57.
 17. Tacon KC, Santos HC, Parente LM, Cunha LC, Lino-Júnior Rde S, Ribeiro-Rotta RF. et al. Healing activity of laser InGaAlP (660nm) in rats. *Acta Cir Bras.* 2011;26(5):373–8.
 18. Marinho RR, Matos RM, Santos JS, Ribeiro MA, Smaniotto S, Barreto EO. et al. Potentialized anti-inflammatory effect of combined 780 nm and 660 nm low level laser therapy on the experimental laryngitis. *J Photochem Photobiol B.* 2013;121:8693.
 19. Mendez TM, Pinheiro AL, Pacheco MT, Nascimento PM, Ramalho LM. Dose and wavelength of laser light have influence on the repair of cutaneous wounds. *J Clin Laser Med Surg.* 2004;22(1):19–25.
 20. Pires D, Xavier M, Araújo T, Silva JA Jr, Aimbire F, Albertini R. Low-level laser therapy (LLLT; 780 nm) acts differently on mRNA expression of anti- and pro-inflammatory mediators in an experimental model of collagenase-induced tendinitis in rat. *Lasers Med Sci.* 2011;26:85–94
 21. Rodrigo SM, Cunha A, Pozza DH, Blaya DS, Moraes JF, Weber JB. et al. Analysis of the systemic effect of red and infrared laser therapy on wound repair. *Photomed Laser Surg.* 2009;27(6):929–35.
 22. Pinheiro AL, Pozza DH, Oliveira MG, Weissmann R, Ramalho LM. Polarized light (400-2000nm) and non-ablative laser (685nm): a description of the wound healing process using immunohistochemical analysis. *Photomed Laser Surg.* 2005;23(5):485–92.
 23. Gál, P., Vidinský, B., Toporcer, T., et al. (2006). Histological assessment of the effect of laser irradiation on skin wound healing in rats. *Photomed. Laser Surg.* 24, 480–488.
 24. Rabelo, S.B., Villaverde, A.B., Nicolau, R., Salgado, M.C., Melo Mda, S., Pacheco, M.T. (2006). Comparison between wound healing in induced diabetic and nondiabetic rats after low-level laser therapy. *Photomed. Laser Surg.* 24, 474–479
 25. Reddy, G.K., Stehno-Bittel, L., Enwemeka, C.S. (2001). Laser photostimulation accelerates wound healing in diabetic rats. *Wound Repair Regen.* 9, 248–255
 26. Al-Watban, F.A., Zhang, X.Y. (1999). The acceleration of wound healing is not attributed to laser skin transmission. *Laser Therapy.* 11, 6–10.
 27. Mendez, T.M., Pinheiro, A.L., Pacheco, M.T., Nascimento P.M., Ramalho L.M. (2004). Dose and wavelength of laser light have influence on the repair of cutaneous wounds. *J. Clin. Laser Med. Surg.* 22, 19–25. of laser application. The most effective healing of a proximal wound was verified
 28. Pinho de Oliveira, R.A. (2002). Efeitos da fototerapia na cicatrização de feridas cirúrgicas produzidas com o laser de CO2 [thesis]. Porto Alegre (RS): Faculdade de Odontologia da PUCRS.
 29. Rochkind, S., Rouso, M., Nissan, M., Villarreal, M., Barr-Nea, L., Rees, D.G. (1989). Systemic effects of low-power laser irradiation on the peripheral and central nervous system, cutaneous wounds, and burns. *Lasers Surg. Med.* 9, 174–182
 30. Renata Saltiel Machado 1, Suane Viana 2, Graciele Sbruzzi 3 Low-level Laser Therapy in the Treatment of Pressure Ulcers: Systematic Review 2017 May;32(4):937-944. doi: 10.1007/s10103-017-2150-9. Epub 2017 Jan 23.
 31. Altan, A. B.; Bicakci, A. A.; Mutaf, H. I.; Ozkut, M. & Inan, V. S. The effects of low-level laser therapy on orthodontically induced root resorption. *Lasers Med. Sci.*, 30(8):2067-76, 2015