Effect of Radiation Therapy on Wound Healing

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Abstract- Many studies were carried out to improve sophisticated approaches to accelerate wound healing processes and also, wound healing is extensively discussed in the medicinal literature. In this study, we aimed to investigate the wound healing of mice with visible radiation (630 nm). In this study, 28 male mice were used and randomly divided into 4 groups. All samples were subjected to a 6 mm skin incision. Four groups were tested as following 10 minutes (First group), 20 minutes (Second group), 30 minutes (Third group) light exposure and no light radiation (control group), respectively. Wound contraction was analyzed by taking photos and Image J software. All mice were sacrificed, and samples were evaluated by Hematoxylin and Eosin staining after one week. The results demonstrated that the experimental group with 20 min.light irradiation had a much faster effect of wound healing than the 10, 30 minute and control group. The results showed that 20 minutes of light exposure group was the best treatment.

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Keywords: Wound healing; Radiation therapy; Skin; Mice

Introduction

Skin is the largest organ and is made by three layers; Epiderm, Derm, and Hypoderm. Derm and Hypoderm include blood vessels and lymphatic vessels, fecal glands, glandular and other ganglia,and hair follicles. Wound healing is a complex process,but it is known as an organized phenomenon. Certain processes happen in wound healing such as reconstruction, migration, reproduction of parenchymal cells and connective tissue cells. Wound healing demonstrates a dynamic physiological approach which included by a lot of agents. The process can be divided into four steps: hemostasis, inflammation, proliferation, and finally remodeling (1). Wound healing is completed by the activity of numerous cells with inflammatory phases, cell proliferation, and collagen synthesis which are followed by regeneration phase of wound structure, collagen maturationof new epidermis, fibroblasts and newly formed vessels. Totally, a dynamic coordinated cascade of cellular and molecular actions which encompasses four overlapping phases: hemostasis, inflammation, granulation tissue formation, and remodeling (2,3). In pathologic circumstances such as a non-healing chronic wound, this efficient and orderly process gets hampered which may be the consequences of dysregulated or stagnant inflammation, enhanced free radicals mediated damage, decreased angiogenesis and reduced collagen accumulation, which inhibit the normal processes of wound healing (4,5). Photodynamic therapy using blue light has been shown to considerably decrease acne lesions in studies on mild to moderate, inflammatory, and pustular acne when irradiating over eight to 10 treatments (6). Visible light (630 nm) is considered as one of the available methods. The most important mechanisms of this method are in shortening of the inflammatory stage, accelerating of cell proliferation and increased blood supply. Ultimately, it accelerates wound healing. The effect of radiation (630 nm) has been shown to be effective in treating of the wound healing process, inflammation phase, reduces edema and increases blood flow in the angiogenesis of the wound and promotes the proliferation of collagen cells in the scar tissue by the visible light of 630 nm. In this research, visible light (630 nm) were investigated on 6 mm skin incision wound in mice.

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Materials and Methods

Animals

In a randomized clinical trial, 28 male mice (average weight: 25-30 g, average age: 3 months) were randomly divided into 4 equal groups. 10 minutes (First group), 20 minutes (Second group), 30 minutes (Third group) light exposure and no light radiation (control group). They were all maintained in a sheltered environment (temperature: 20-25˚ C and humidity: 65-75%) under the supervision of a veterinarian. During the experiment, the mice were fed with usual mice chow and tap water and each group was kept in a separate cage. Studies on all groups were done at the same time. The rats were anesthetized by xylazine (10 mg/kg) and ketamine hydrochloride (50-100 mg/kg) (7-10).

Incision wound model

In general, anesthesia induced by intramuscular administration of ketamine (ketamine 10%, 50 mg/kg) and xylazine (2%, 10 mg per kilogram), and then, a small incision (6 mm) was made. All mice were tested in four groups; 10 minutes (First group), 20 minutes (Second group), 30minutes (Third group) light exposure of 630 nm and no light radiation (control group). The wound healing and the size of the wounds were examined by the Image J software and also, Tensiometer test was done (7-10).

Histological observations

All mice were sacrificed and sent to the lab for histological study. The samples were stained with Hematoxylin and Eosin staining on 7 days (10-13).

Statistical analysis

Repeated measures ANOVA test was used to detect any overall differences between related means. In the statistical test, a $P<0.05$ was considered being significant.

Results

The results of descriptive analyses are shownin table 1. There was a substantial main effect for time (Table 1 and Figure 1), Wilks Lambda=0.29, F(6,19)=104.89, $P<0.001$, Partial eta squared=0.89, with four groups showing a reduction in the size of injury across the seven time periods.

Table 1. Measurement of treatment size between four groups across the seven time periods

Time period	Mean(SD)			
	10 min	20 min	30 min	Control
Day 1	6(0)	6(0)	6(0)	6(0)
Day 2	5.39(0.37)	4.74(1.12)	4.69(0.23)	5.37(0.53)
Day 3	4.83(0.73)	3.60(0.70)	4.18(0.38)	4.86(0.64)
Day 4	4.42(0.79)	3.03(0.82)	3.95(0.28)	4.21(0.52)
Day 5	3.34(0.40)	1.64(1.69)	2.45(0.53)	3.46(0.58)
Day 6	2.60(0.66)	1.09(1.19)	2.11(0.69)	3.05(0.66)
Day 7	1.83(0.99)	0.63(0.94)	1.45(0.82)	2.84(0.74)

The main effect comparing the three types of intervention and control group was significant, F(3.24)=9.7, $P<0.001$, partial eta square=0.54, suggesting that there is difference in the effectiveness of the four groups which 10, 20, 30 min radiation had better-wound healing in comparison with control group (Figure 1).

Post hoc analysis (LSD test) showed that 20 min radiation was the best time for wound healing in comparison with 10 min ($P<0.001$) and 30 min radiation ($P=0.002$). The results of wound healing are shown in figure 2.

The results revealed that there was a considerable in epithelialization in the 20 minutes (Second group) light exposure in comparison with the other groups which are shown in figure 3. A significant increase was observed in the wound elongation in the 20 minutes light radiation which indicated the activity of the fibroblast in producing of collagen fibers

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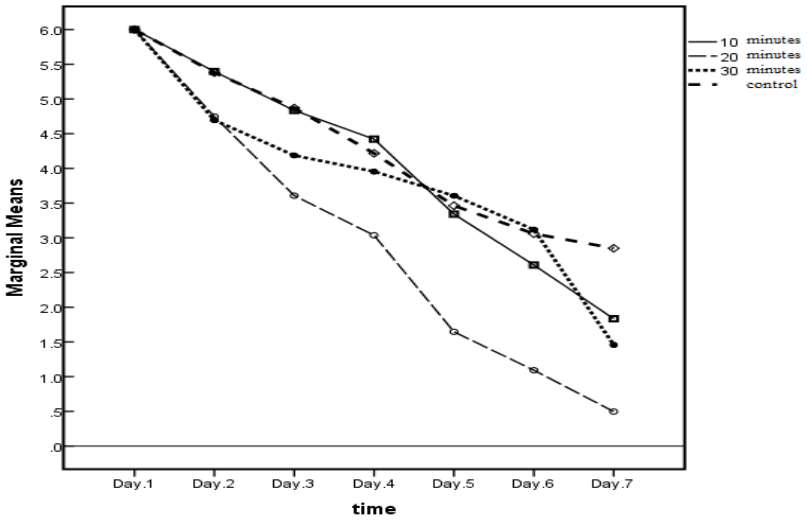

Figure 1. Comparison of wound healing between four groups

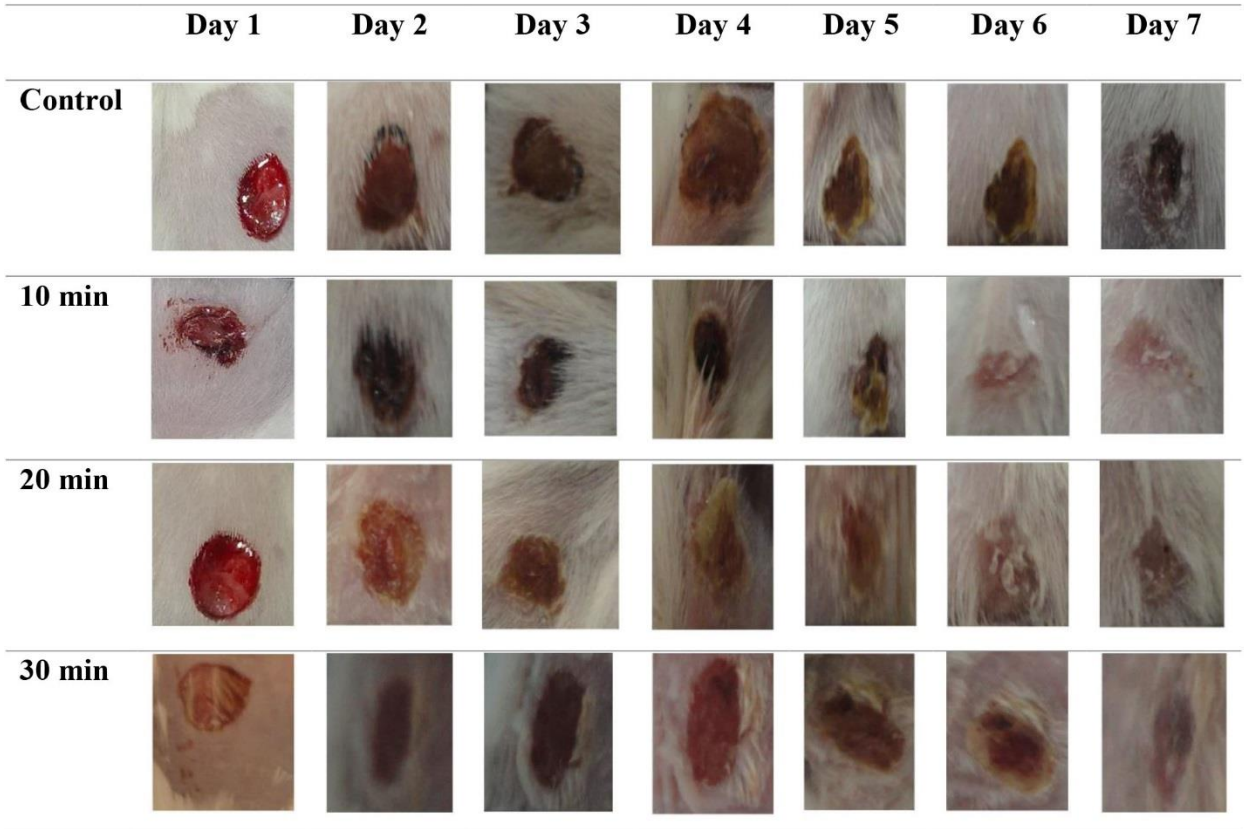

Figure 2. Treatment between four groups

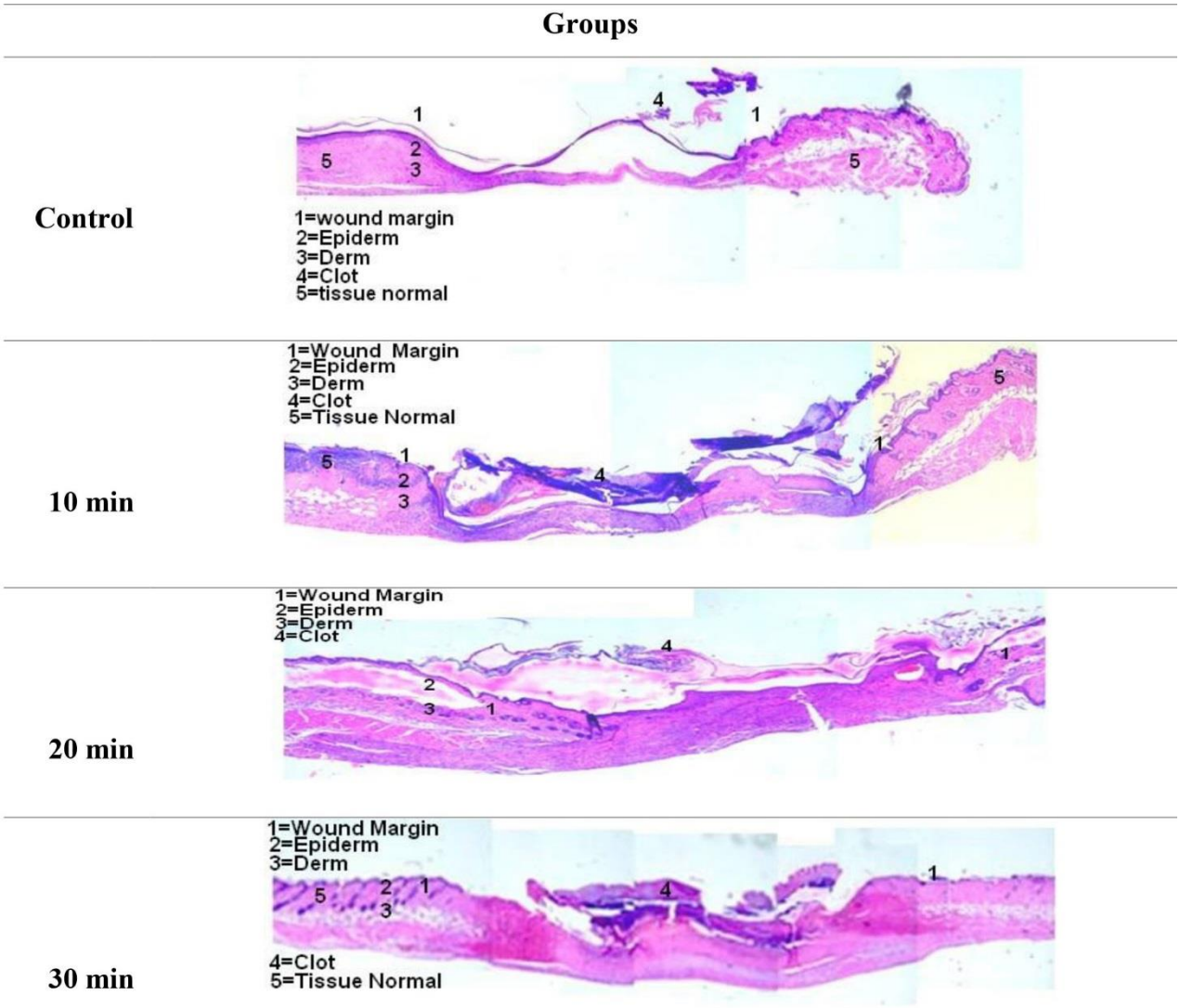

Figure 3. Histological Epithelization (×40)

Discussion

Most people can recover from the wound without serious health consequences, depending on the cause and degree of injury. More serious wound need direct emergency medical care to avoid complications and death. Based on the results, the percentage of wound healing was considerable during the first week in the groups. Wound contraction requires to migration and proliferation of the epithelial cell. While, the mechanical properties of the wound tissue are related to the proliferation of fibroblasts, Collagenization, maturation of collagen fibers, the formation of the subcutaneous tissue of the connective tissue and the contraction of collagen fibers which are investigated in various studies. Fernández-Guarinoand colleagues evaluated clinical photodynamic therapy in patients with actinickeratosis, and the improvement of lesions was obtained in face part with lower treatments by using imaging fluorescence. They concluded that the best treatment was the back of hands. The fluorescence area and the severity of it strongly suggested in the treated areas a significant correlation with the development of clinical response. Goldberg and colleagues researched aboutthe combination ofblue (415 nm) and red (633 nm) LED phototherapy in the treatment of mild to severe acne vulgaris and found that combinationof blue and red LED therapy to have excellent potential in the treatment of mild to severe acne. Treatment appears to be both pains- and side-effect-free (14). Papageorgiou and colleagues

researched the effects of a blue and red light combination treatment in a randomized study of 107 patients with mild to moderate acne. Results showed a 76% reduction in inflammatory lesions in the combination group (6). Frangez and colleagues investigated the effect of LED on blood microcirculation during chronic wound healing in diabetic and non-diabetic patients,and they found that phototherapy with LED was shown to be an effective additional treatment method for chronic wounds in diabetic and non-diabetic patients (15). Solmaz and colleagues researched in photobiomodulationof wound healing via visible and infrared laser irradiation and they found that 635 nm laser irradiations of low energy densities had stimulatingeffects in terms of cell proliferation in vitro and mechanical strength of incisions in vivo. However, 809 nm laser irradiations at the same doses did not have any positive effect (16). Gaurav and colleagues investigated the Photobiomodulation with Pulsed and Continuous Wave Near-Infrared Laser (810 nm, Al-Ga-As) Augment Dermal Wound Healing in Immunosuppressed Rats,and the found that healing efficacy of 10 Hz pulsed mode was more promising than CW and 100 Hz pulsed mode (17). Laser therapy can reduce the inflammation which causes the acceleration of proliferation, wound contraction and biomechanical wound improvement. In this study, we showed that light could increase the proliferation of cells and rapid wound contraction in the first week.Furthermore, light could increase fibroblasts in comparison with the control group,and it can be concluded that all three types of radiation (10, 20, 30 minutes) led to rapid wound contraction and increase the parameters stiffness, strain, stress elasticity of the tissue. Radiation could produce more collagen in three types of radiation (10, 20, 30 minutes) by increasing of fibroblasts in comparison with the control group. In this research, histological epithelialization tests showed that 20 min radiation therapy was the best treatment and also, the results showed that wound contraction was faster than other groups. These results showed that radiation therapy could be used for healing of burn wounds.

It can be concluded that radiation therapy usage had a significant effect on epithelialization and in wound healing. Moreover, the time of wound contraction was significant in the second group (630nm, 20 minutes). These results recommend that it can be used for wound healing.

References

1. Boateng JS,Matthews KH, Stevens HN, Eccleston GM. Wound healing dressings and drug delivery systems: a review. J Pharm Sci 2008;97:2892-3.
2. Dickinson LE,Gerecht S. Engineered BiopolymericScaffolds for Chronic Wound Healing. Front Physiol 2016;7:341.
3. Singer AJ, Clark RA. Cutaneous wound healing. N Engl J Med 1999;341:738-46.
4. Gupta A, Manhas N, Raghubir R. Energy metabolism during cutaneous wound healing in immunocompromised and aged rats. Mol Cell Biochem 2004;259:9-14.
5. Sharif F, Steenbergen PJ, Metz JR, Champagne DL. Long-lasting effects of dexamethasone on immune cells and wound healing in the zebrafish. Wound Repair Regen 2015;23:855-65.
6. Papageorgiou P, Katsambas A, Chu A. Phototherapy with blue (415 nm) and red (660 nm) light in the treatment of acne vulgaris. Br JDermatol 2000;142:973-8.
7. Amini M, Kherad M, Mehrabani D, Azarpira N, Panjehshahin MR, Tanideh N. Effect of Plantago major on burn wound healing in ratJApplAnim Res 2010;37:53-6.
8. Gupta Singh O, Bhagel PS, Moses S, Shukla S, Mathur RK.Honey dressing versus silver sulfadiazene dressing for wound healing in burn patients: A retrospective study. J CutanAesthetSurg 2011;4:183-7.
9. Somboonwong J, Thanamittramanee S, Jariyapongskul A, Patumraj S.Therapeutic effects of Aloe vera on cutaneous microcirculation and wound healing in second degree burn model in rats. J Med Assoc Thai 2000;83:417-25.
10. Chen J, Han CM, Lin XW, Tang ZJ, Su SJ. Effect of silver nanoparticle dressing on second degree burn wound. Zhonghuawaikezazhi 2006;44:50-2.
11. Babavalian H, Latifi AM, Shokrgozar MA, Bonakdar S, Shakeri F, Tabyanian H. Healing Effects of Synthetic and Commercial Alginate Hydrogel Dressings on Wounds: A Comparative Study. Trauma Mon 2016 [Epub ahead of print].
12. Shakeri F, TebyanianH, Karami A, Babavalian H, Tahmasbi MH. Effect of Topical Phenytoin on Wound Healing. Trauma Mon 2016;22:e35488.
13. Subrahmanyam M. A prospective randomised clinical and histological study of superficial burn wound healing with honey and silver sulfadiazine. Burns 1998;24:157-61.
14. Goldberg DJ, Russell BA. Combination blue (415 nm) and red (633 nm) LED phototherapy in the treatment of mild to severe acne vulgaris. J Cosmet Laser Ther2006;8:71-5.
15. Frangez I, Cankar K, Ban Frangez H, Smrke DM.The effect of LED on blood microcirculation during chronic wound healing in diabetic and non-diabetic patients—a

prospective, double-blind randomized study. Lasers Med Sci 2017;32:887-94.

16. Solmaz H, Ulgen Y, Gulsoy M.Photobiomodulation of wound healing via visible and infrared laser irradiation. Lasers MedSci 2017;32:903-10
17. Keshri GK, Gupta A, Yadav A, Sharma SK, Singh SB.Photobiomodulation with Pulsed and Continuous Wave Near-Infrared Laser (810 nm, Al-Ga-As) Augments Dermal Wound Healing in Immunosuppressed Rats. PLoS One 2016;11:e0166705.