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Influence of Helium-Neon Laser Photostimulation on Excision Wound Healing in Wistar Rats

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Abstract: The importance of laser photostimulation is 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 in excision wounds using excision wound model in Wister rat model. The circular wounds were created on the dorsum of the back of the animals. The animals were divided into two groups. The experimental group (n = 12) wound was treated with 632.8 nm He-Ne laser at a dose of 2.1J cm^{-2} for five days a week until the complete healing. The control group was sham irradiated. The parameters studied were wound area, period of epithelization and hydroxyproline. Significant increase in the hydroxyproline content (p<0.001) and reduction in the wound size (p<0.001) was observed in study group when compared to controls. The significant epithelization (p<0.001) was noticed. The experimental wounds were, on average, fully healed by the 15th day, whereas the control group healed, on average by 22nd day. Wound contraction together with the hydroxyproline and experimental observations suggested that low intensity Helium-Neon laser photo stimulation facilitates the tissue repair process by accelerating collagen production in chronic wounds.

Key words: Laser photostimulation, wound healing, hydroxyproline, autoCAD RL, 14, experimental observation and excision wound

INTRODUCTION

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]. Chronic wounds are slow, non-healing wounds that can last for weeks despite adequate and appropriate care. Such wounds are difficult and frustrating to manage. Wound healing is an enigmatic and debilitating complication and poses a serious challenge in clinical practice. The exact pathogenesis of the poor wound healing in chronic 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 wound healing and tissue repair remains poorly understood. The purpose of this study is to find out the photo stimulatory effect of HeNe Laser on excision wound healing by comparing the biochemical analysis and wound area measurement in Wister rat's models.

MATERIALS AND METHODS

Animals: Healthy inbred albino Wistar strain male rats weighing 180-220 g were used for the study. They were individually housed and were fed with pellet food supplied by gold Mohur Lipton India Ltd. Animals were periodically weighed before and after the

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experiment. The rats were anaesthetized prior to and during infliction of the experimental wounds. The surgical interventions were carried out under sterile conditions using ketamine anaesthesia (10 mg kg^{-1}). Animals were closely observed for any infection and those which showed signs of infection were separated and excluded from the study and replaced. The study was approved by the Ethics Committee for animal experimentation, Manipal Academy of Higher education.

Excision wound model: Animals were anaesthetized prior to and during creation of the wounds. The rats were inflicted with excision wounds as described by Morton and Malon[14]. The dorsal fur of the animals was shaved with an electric clipper and the anticipated area of the wound to be created was outlined on the back of the animals with methylene blue using a circular stainless steel stencil. A full thickness of the excision wound of circular area 400 mm^2 and 2 mm depth was created along the markings using toothed forceps, a surgical blade and pointed scissors. The entire wound was left open[15]. The animals were divided into two groups of 12 each. Control animals were sham irradiated. The experimental group (n = 12) wound was treated with 632.8 nm He-Ne laser at a dose of 2.1J cm^{-2} for five days a week until the complete healing. The wound closure rate was assessed by tracing the wound on days 1, 5 and 10 and 15 post-wounding using transparency paper and a permanent marker. The wound areas recorded were measured using AutoCAD RL 14 Computer (AutoDesk inc. USA) Method.

Laser therapy schedule: The low energy He-Ne laser provides infrared rays in the wavelength of 632.8 nm by continuous mode. An average power of 2.1 cm^{-2} was provided through a fiber optic delivery system over the wound for 5 days a week until complete healing.

Estimation of hydroxyproline: On day 15, the tissue by 1 cm wide by approximately 2 cm in length were collected from each animals for analysis. The tissue was dried in an oven at 60ºC and the dry weight was noted. The acid hydrolysate of the dry tissue was used for the determination of hydroxyproline. To 0.1 mL acid hydrolysate added 0.4 mL of deionised water, 1.0 mL of 2.5 N NaOH, 1.0 mL 0.01 M $CuSO_4$ and 1.0 mL 6% hydrogen peroxide. Tubes were covered with marbles and boiled for 15 min at 80°C. Then 2.0 mL para-methylaminobenzaldehyde (Ehrlicks reagent) and 4.0 mL of 3 N H_2SO_4 was added. Incubated at 80°C for 15 min and the absorbance was read at 540 nm using spectrophotometer.

Experimental observation: The wounds were observed for contraction (healing), granulation and epithelization in both the groups until complete closure of the wound. The rate of healing and the mean wound-healing time was calculated. For epithelization analysis the margin of the wound and the limit of epithelization were marked at the site of maximum and minimum epithelization, it was measured by vernier caliper and the mean value of epithelization was noted.

RESULTS AND DISCUSSION

Table 1 shows the effect of low intensity He Ne laser therapy irradiation on the wound area, hydroxyproline and epithelization concerned with the process of wound healing. The wound area measurement by AutoCAD RL 14 computer analysis showed significant reduction in wound size of the study group ($p<0.001$) as compared to control group. Significant increase in the hydroxyproline content ($p<0.001$) was observed in study group when compared to controls. The significant epithelization ($p<0.001$) was noticed. The experimental wounds were, on average, fully healed by the 15th day, whereas the control group healed, on average by 22nd day.

Wound healing is a complex and dynamic process of restoring cellular structures and tissue layers in damaged tissue as closely as possible to its normal state. Wound contracture is a process that occurs throughout the healing process, commencing in the fibroblastic stage whereby the area of the wound undergoes shrinkage. It has 3 phases; inflammatory, proliferative and maturational and is dependent upon the type and extent of damage, the general state of the host's health and the ability of the tissue to repair. The inflammatory phase is characterized by hemostasis and inflammation, followed by epithelization, angiogenesis, granulation tissue formation and collagen deposition in the proliferative phase. In the maturational phase, the final phase of wound healing the wound undergoes contraction resulting in a smaller amount of apparent scar tissue. The present study showed that the low

Table 1: Effect of He-Ne laser therapy irradiation on wound healing

Parameter	Control group	Experimental group
Woundarea (mm^2)		
Day 1	333.78±8.03	333.78±8.165
Day 5	256.78±8.99	192.56±0.26
Day 10	203.56±1.15	33.56±1.15
Day 15	91.56±5.77	3.54±5.77**
Epithelization	1.41±0.51	3.75±0.96**
Hydroxyproline(mg/g tissue)	17.50±0.28	61.70±0.70**

**: $p<0.001$

intensity laser irradiation is reported to have anti-inflammatory activity and proliferative 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.

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[16,17]. Clinically laser photo stimulation has been used to treat pain, tropic ulcers indolent wounds of diverse etiologies, bone repair and soft tissue injuries[18-20]. There are however, studies reporting conflicting results on the effects of laser photo stimulation on wound healing and its constituents[21-23]. The reason for the discrepancies might be found in the great variety of treatment protocols and animal or cell culture models used. Moreover, there is evidence that the effect of low energy laser stimulation also depends on the physiological state of the cell or tissue at the moment of exposure[24, 25]. It is possible that the laser therapy has an optimal effect under conditions of impaired healing where the enhancement of healing can be measured.

This study shows that low energy laser photo stimulation facilitates the wound repair process as evidenced by the wound area measurement and biochemical analysis. Both area measurement and biochemical findings indicate that significant improvements occurred in the healing process following treatment. The results of the study show that the production of collagen in excision wounds can be modulated by laser treatment. This effect may involve a variety of photo stimulating mechanisms[26]. It is mainly due the fact that the laser energy at certain frequencies can modulate cell proliferation and release the growth factors from fibroblasts. The other mechanism of photostimulation was that the mitochondria are the photo acceptors for light energy. The absorption of energy by the respiratory chain may cause oxidation of NADH, producing changes in the redox status in mitochondria and cytoplasm, Activation of electron transport chain results in an increase in the electrical potential across the mitochondria membrane, an increase in the ATP pool and finally the activation of nucleic acid synthesis.

In an effort to provide a more clinically relevant wound healing model, we investigated the effect of laser photo stimulation on excision wounds. The biochemical analysis and wound area measurement results from this study clearly indicate that low energy photo stimulation with He-Ne laser is beneficial in promoting wound healing.

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