Effect of Low-Power Helium-Neon Laser Radiation on the Healing of Deep Second-degree Burns in Rats

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Abstract

Received 14/Jul/2002, Accepted 10/Feb/2003

Introduction: This paper presents the results of a study on the effects of two different doses of low-power laser irradiation on healing of deep second degree burns.

Material and Methods: 60 rats were randomly allocated to one of four groups. A deep second-degree burn was inflicted in each rat. In the control group (CG) burns remained untreated; in Groups LG1 and LG2 the burns were irradiated with low-power Helium Neon laser with energy densities of 1.2 J/cm^2 and 2.4 J/cm^2 respectively. In the fourth group (G4) the burns were treated topically with 0.2% nitrofurazone cream. The response to treatments was assessed histologically at 7, 16 and 30 days after burning and microbiologically at Day 15.

Results: The number of macrophages and the depth of new epidermis was significantly less in the laser treated groups compared to control and nitrofuorazone treated groups. Staphylococcus epidermidis was found in the wounds of all rats in the laser treated groups.

Conclusion: Irradiation of deep second-degree burn with low-power laser produced no beneficial effects on healing of burns.

Key words: Second-degree burn, Laser, Histology, Microbiology, Rat

Introduction

Thermal burns are less common than other forms of trauma but produce more severe physiological stresses than other forms of traumatic injury. It is estimated that annually two million people suffer burns in the USA (1). Low-power lasers have recently been investigated for stimulation of cell activities involved in the wound healing process. A number of effects have been claimed; these include increase of ATP production (2), increase in mitochondrial membrane potential (3) and its activity (4), transformation of fibroblasts into myofibroblasts (5) and proliferation of keratinocytes in vitro (6). In laboratory animals, biostimulation of the wound healing process by laser irradiation has been reported to include, stimulation of fibroblast proliferation, increased reepithelialization and collagen synthesis and granulation tissue formation, accelerated wound closure, improved tensile strength of scars and faster healing of burns (7-11). Other studies have reported a bactericidal effect of low-power laser irradiation in vitro (12-14). However a number of investigators found no improvement in wound healing and healing of burns (15-20). Clearly there is no consensus on the effects of low-power laser treatment in wound healing. Because burn healing studies have used various types of low-power lasers with different wavelengths, laser power and stimulation doses no clear recommendation can be made regarding the type of laser and wavelength. There have been no published reports on the effect of low-power He-Ne laser with a wavelength of 632.4 nm on deep second degree burns. Therefore, the aim of the present study was to examine the influence of laser irradiation with a 632.4nm/10 mW low-power He-Ne laser on the healing histo-pathology and microbial flora of deep second degree burns in rats.

Material and Methods

Sixty male adult wistar rats weighing 250±30 g kept in separate cages and fed ad libitum were used in the experiment. On day zero, each rat was anesthesized by intramuscular injection of 50 mg/kg ketamine hydrocholoride, with 5 mg/kg diazepam and held in a special box which had a 3 × 3 cm hole. The back of each rat was exposed for three seconds, via the 3x3 cm hole, to the external lip of a cylinder 22 mm in diameter connected to a source of boiling water. Histological examination showed that the epidermis and most of the of dermis were burned.

The animals were randomly divided into four groups. Control group rats (CG) were left untreated. The rats in Groups LG1 and LG2 were exposed each day to 1.2 and 2.4 J/cm^2 low-power laser light respectively. The specifications of the laser used (made by Iranian Atomic Energy Agency, Tehran Iran) are shown in Table 1. To administer laser treatment, the tip of the laser source was in light contact with the surface of the burn and directed perpendicularly to the target tissue for the designated time (21). The rats of the fourth group (G4) were treated daily with topical 0.2 % nitrofurazone cream (Tehran daru co., Tehran, Iran). Of the 15 rats in each group 5 were killed by choloroform 7 days after burning, 5 16 days after burning and the remainder 30 days after burning. A sample for histological examination was excised from the burn bed of each rat, fixed in formal saline and embedded in paraffin blocks. Sagittal sections were cut and stained with haematoxylin and eosin. Ten zones from each sample were examined morphometerically using a calibrated ocular on a Nikon light microscope at a magnification of 400 times for counting fibroblasts, macrophages, neutrophils and blood vessels sections.

The depth of new epidermis and new dermis (granulation tissue) were measured at 10 points. The data were subjected to analysis of variance (ANOVA) and expressed as means ± SD. Multiples comparisons were performed by least significant difference (LSD) test. $P < 0.05$ was considered statistically significant.

At day 15 microbiological samples were taken from the burn sites of all rats, cultured and tested for identification of Staphylococcus epidermis and subtilis and staphylococcus aureus and Pseudomonas aeruginosa using the methods originally described by Fingold and Martin (22), Baron and Fingold (23). and Brook et al (24). Data for each organism were compared between groups of rats by Exact Fisher test. A further comparison was made between treatment groups for organisms assumed to be non-pathogenic

(Class 1- Staphylococcus epidermis and subtilis) and for organisms assumed to be pathogenic (Class2- Staphylococcus aureus and Pseudomonas aeruginosa). Data of classes one and two were statistically compared using Mann Whitney U test. $P<0.05$ was considered statistically significant.

Results

Statistical analysis of histological features (Figs 1, 2 and 3) showed that there were significant differences between groups in macrophage counts at day 16 ($F=15.189$, $Sig= 0.000$; Fig1). Analysis by LSD, revealed significant differences between the control group (CG) and laser treated groups (LG1 and LG2).

There were also significant differences between groups in depth of new epidermis at day 30 ($F=7.269$, $Sig= 0.000$; Fig 3) and LSD analysis revealed significant differences between the fourth group (G4) and other groups and between the control group (CG) and first laser group (LG1).

Statistical analysis of the incidence of microbial flora (Table 2) showed that there were significant differences between groups. The incidence of Staphylococcus epidermidis differed significantly between the control group (CG) and the fourth group(G4) and between the first laser group (LG1) and the fourth group (G4) ($P=0.048$). The incidence of the first class of bacteria differed significantly between the fourth group and other groups ($P= 0.014$).

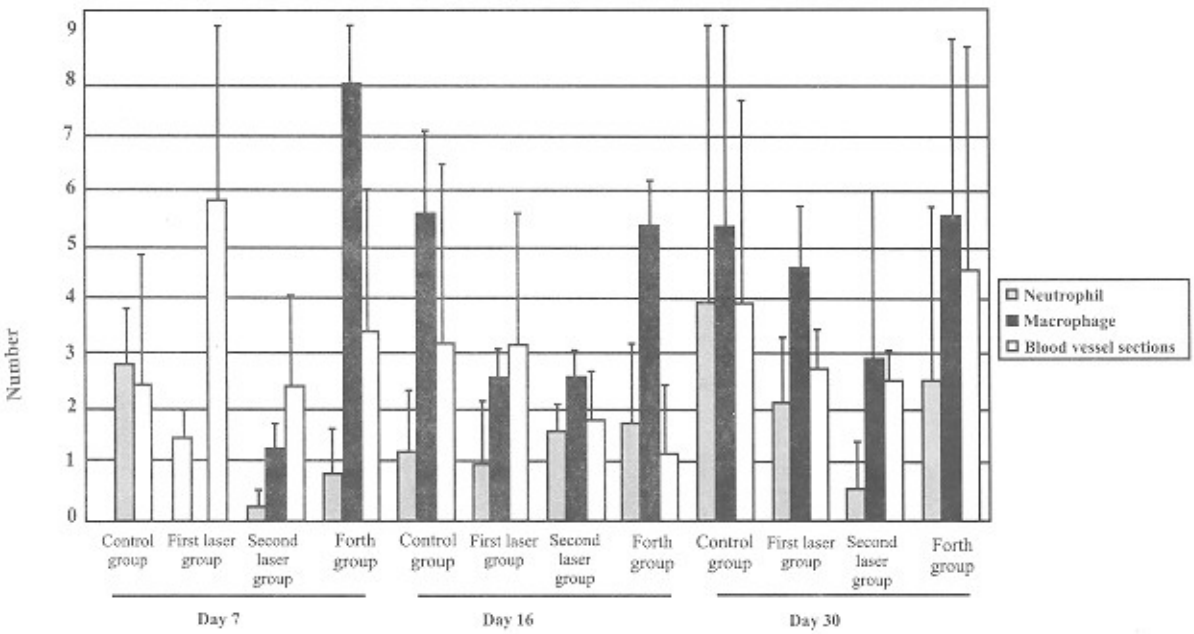

Fig 1: Mean ± SD of neutrophils and macrophages and blood vessel sections in ten zones of histological slides of rats of study groups at sequential intervals.

Analysis of variance showed that there were significant differences between macrophages of study group at day 16 ($F= 15.189$, $Sig=0.000$) and by doing least significant differences method it were revealed that significant differences were between control and laser groups.

Table 1: Specifications of the low-power laser used

Laser source: 10 mW Helium - Neon laser tube
Wavelength: 632.8 nm
Frequency: continuous
Timing: 120 sec/cm^2/day for first laser group
240 sec/cm^2/day for second laser group
Technique of radiation: Grid Technique (21)

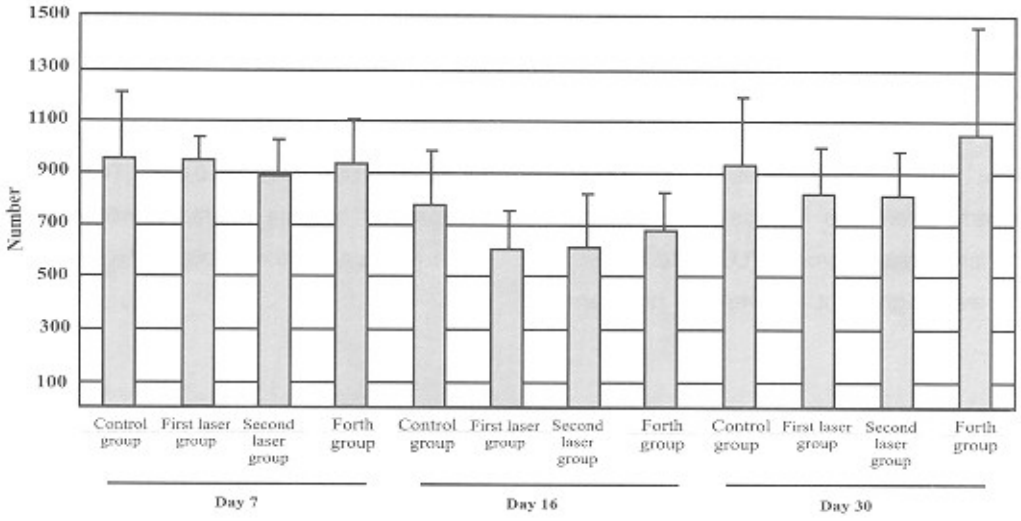

Fig 2: Mean ± SD of fibroblast in ten zones of histological slides of rats of study groups at sequential intervals.

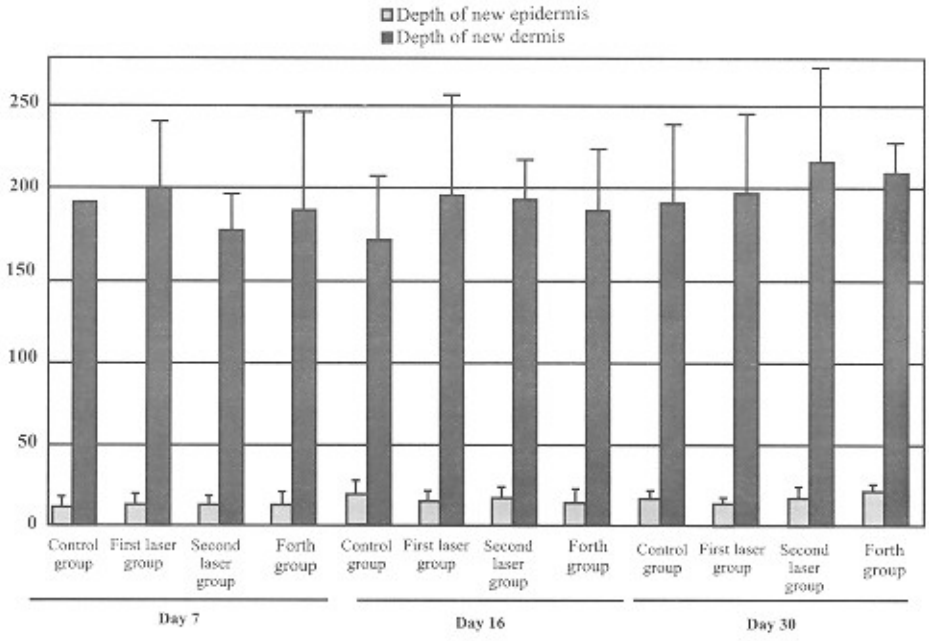

Fig 3: Mean ± SD of depth of new epidermis (μ) and new dermis (μ) of rats of study groups at sequential intervals.

Analysis of variance showed that there were significant differences between depth of new epidermic of study groups at day 30(F= 7.269, Sig=0.000) and by doing least significant method it were revealed that significant differences were between fourth group and other groups.

Table 2. Numbers of rats from which bacteria were cultured

Group	n	Staphylococcus Epidermidis	Subtilis	Staphylococcus aureus	Pseudominas areuginosa
Control (CG)	5	5	1	0	0
First laser (LGI)	5	5	0	0	0
Second laser (LG2)	5	2	1	0	0
Fourth Group (G4)	5	1	0	2	0

There were Significant Differences:

- Between staphylococcus epidermidis of control group and fourth group, first laser group and fourth group ($p=0.048$).
- Between first class of bacteriae of fourth group and other groups ($p=0.014$).
- Between second class of bacteriae of nitrofurazone group (G4) and other groups ($p=0.048$).

The incidence of the second class of bacteria differed significantly between the fourth group and other groups ($P= 0.048$).

Discussion

Findings from the current study demonstrated that laser irradiation decreased the number of macrophages at day 16 in comparison to control group and decreased the depth of new epidermis at day 30 in comparison with control and nitrofurazone treated groups. These findings indicate that irradiation of burns with low-power laser produces no beneficial effect on healing of burns and are in agreement with those demonstrated previously by Cambier et al (18) and Schlager et al studies (19, 20). However there are several reports on positive effects of laser on wound healing (8-10). The reasons for this diversity may be the different milieu and microenvironments associated with cutaneous wounds (25) and burns (26) and variations in the application of laser light. It is suggested that in the present study healing of the burns was not accelerated by exposure to laser light because there were no normal cells in the burn area. This may not have been apparent when Schlager et al (19) and Camber et al (18) evaluated healing of burns macroscopically. Schlager et al in another study evaluated healing of burns both macroscopically and histologically. Cambier et al used a Helium Neon laser (0.21 J/cm^2) and a Gallium-Arsenide laser (0.75 J/cm) in o^{2}ne of two burns (surface, 2 cm^2) that were inflicted in each rat. Schlager et al in one study used 670 nm low-power laser (2 J/cm^2) with 250 mW output, two different lasers with wavelengths of 635 nm and 690 nm, and energy density of 2 J/cm^2 in both cases, and outputs of 30 mW and 12 mW respectively. Schlager et al inflicted two burns (surface 1.54 cm^2) in each rat and irriadiated one of the burns. In spite of the clinical importance of burn depth (27), in these studies (18 20) the depth of burn is unknown and no explanation of the negative results was provided. The results of the present study and of Cambier et al (28) are similar.

However there have been some favourable findings on the effect of laser light on healing of burns. Mester et al (10), using a ruby laser (694.3 nm) with 0.2 J/cm^2, 1 J/cm^2, 5 J/cm^2 and 10 J/cm^2 energy densities (doses) on third degree burns in mice, found a significant effect of the 1 J/cm^2 dose but not the other doses on reepithelialization. Rochkind and colleagues (11) found accelerated healing of burns after treatment with a He-Ne laser (10 J/cm^2). It is note worthy that both Mester et al and Rochkind and colleagues reported beneficial effects of the laser treatment despite their use of widely differing energy densities (i.e. 1 J/cm^2 versus 10 J/cm^2).

In the present study, topical application of nitrofurazone cream on burns significantly increased the depth of new epidermis at day 30 (Fig 2). Because of the role of epithelialization in covering the wound (29) it seems that this finding is important. However the application of this cream to burns had no other

significant effect on healing. The microbiological examinations showed that laser treatment did not reduce the incidence of bacteria in the burns and the application of nitrofurazone cream did not completely inhibit the growth of Staphylococcus aureus.

Staphylococcous epidermidis is a common inhabitant of human skin (30) and it is not pathogenic in the skin although it can infect native and prosthetic heart valves, catheter and shunts, and prosthetic joints (31). Its presence in laser treated burns can be considered of no clinical importance. In conclusion we found no acceleration of healing of deep second degree burns in healthy rats after treatment with low-power Helium Neon laser irradiations at energy densities of 1.2 J/cm2 or 2.4 J/cm2. It is suggested that future investigations of laser treatment of burns might examine the effect of treatment on common species of bacteria that have been added to the wounds, and the influence of photosensitizing agents on the response.

Acknowledgments

We wish to thank the research department of medical faculty of Shaheed Beheshti University of medical sciences which granted for this study (grant no: 13266) and cell and molecular biology research center of the corresponding university for it's laboratory facilities.

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