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BRIEF REPORT

Effects of equal daily doses delivered by different power densities of low-level laser therapy at 670 nm on open skin wound healing in normal and corticosteroid-treated rats: a brief report

Kamila Lacjaková · Nikita Bobrov · Martina Poláková · Martin Slezák · Martina Vidová · Tomáš Vasilenko · Martin Novotný · František Longauer · Ľudovít Lenhardt · Juraj Bober · Mikuláš Levkut · František Sabol · Peter Gál

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Abstract The optimal parameters for low-level laser therapy (LLLT) for wound healing are still open to discussion. Hence, our study was aimed at comparing the effects of different power densities of LLLT at 670 nm in rats. Four round full-thickness skin wounds were placed on the backs of 16 rats which were divided into two groups (non-steroid and steroid-treated). Three wounds were stimulated daily with a diode laser (daily dose 5 J/cm^2) at different power densities (5, 15 and 40 mW/cm^2, respectively), and the fourth wound served as a control. Six days after surgery all animals were killed and samples removed for histological evaluation. Significant acceleration of fibroblast proliferation and new vessel formation was observed in wounds treated at the selected power densities. No significant differences were found in corticosteroid-treated rats. In conclusion, LLLT with the methodology used improved wound healing in non-steroid rats, but was not effective after corticosteroid-treatment.

Keywords Daily dose of 5 J/cm^2 · Low-level laser therapy · Wound healing · Different power densities

Introduction

It is well known that systemic administration of corticosteroids, which is frequently used as a nonspecific anti-inflammatory therapy in various diseases and in trauma [1],

Kamila Lacjaková and Nikita Bobrov contributed equally to this work.

K. Lacjaková · M. Poláková · M. Vidová
Department of Medical Biophysics,
Pavol Jozef Šafárik University,
040 11 Košice, Slovak Republic

N. Bobrov · F. Longauer
Department of Forensic Medicine, Pavol Jozef Šafárik University,
041 80 Košice, Slovak Republic

M. Slezák · T. Vasilenko · P. Gál (✉)
Department for Biomedical Research,
East-Slovak Institute of Cardiovascular Diseases,
Ondavská 8,
040 11 Košice, Slovak Republic
e-mail: galovci@yahoo.com

M. Novotný
Department of Pathological Physiology,
Pavol Jozef Šafárik University,
040 11 Košice, Slovak Republic

Ľ. Lenhardt · M. Levkut · P. Gál
Department of Pathological Anatomy,
University of Veterinary Medicine and Pharmacy,
041 81 Košice, Slovak Republic

J. Bober
1st Clinic of Surgery, Pavol Jozef Šafárik University and Louise Pasteur Faculty Hospital,
040 11 Košice, Slovak Republic

F. Sabol
Clinic of Heart Surgery,
East-Slovak Institute of Cardiovascular Diseases,
040 11 Košice, Slovak Republic

may lead to poor wound healing due to its catabolic effects in the skin [2]. Although many promising physical methods, such as vacuum-assisted closure [3], treatment with magnetic field pulses [4], light emitting diode irradiation [5] and low-level laser therapy (LLLT) [6, 7], have successfully been used to improve wound healing, corticosteroid-induced wound healing impairment is still a significant problem in clinical practice.

Although, the exact mechanism of action of LLLT in wound healing is still not fully clarified, it has been documented that red lasers at a dose of either 4 or 5 J/cm^2 accelerate wound closure, increase collagen deposition and reduce inflammation during wound healing, and thus positively modulate repair processes [8, 9]. In contrast, in in vitro studies [10, 11] and an in vivo study [12] reductions in the rate of proliferation/healing were observed after increasing the dose to 10 and 20 J/cm^2, respectively. Moreover, do Nascimento et al. showed in a histological study that LLLT at 670 and 685 nm and a dose of 10 J/cm^2 is more effective when a higher intensity is combined with a shorter wavelength or a lower intensity with a higher wavelength [13]. Similarly, we found in our previous study that LLLT at 635 nm and 5 J/cm^2 acts in a power density-dependent manner [9].

Comparing the effects of 665, 675, and 810 nm laser light on the proliferation of fibroblasts and endothelial cells in culture, the highest proliferative activity was observed in cultures stimulated at 665 nm, while irradiation at 810 nm inhibited the proliferation of fibroblasts [14]. Most of the target cells of LLLT are located in the epidermis (epidermal stem cells) and in the upper parts of the dermis (fibroblast, macrophages and endothelial cells) [15, 16]. Accordingly, red lasers may be more suitable for skin treatment than infrared lasers due to their poor depth of tissue penetration [17].

Since the InGaAlP laser (670 nm) is commonly used for LLLT in clinical practice, we decided to assess its effects in our experimental study. Hence, in the present experiments we compared the effects of different power densities but equal daily doses from this laser on the healing of excisional skin wounds in non-steroid and corticosteroid-treated rats using histological evaluation.

Materials and methods

Animal model

This experiment was approved by the Ethics Committee of the Faculty of Medicine of P. J. Šafárik University and by the State Veterinary Administration of the Slovak Republic.

Sprague-Dawley rats (n=16) at 10 months of age weighing 500–550 g were randomly divided into two groups of eight animals: a non-steroid group and a steroid-treated group. Under general anaesthesia (ketamine 40 mg/kg, xylazine 15 mg/kg, tramadol 5 mg/kg), four round full-thickness skin wounds, 4 mm in diameter, were placed on the back of each rat. Wounds were placed in the corners of a square of side 5 cm.

Animals in the steroid-treated group received a bolus dose of 20 mg/kg methylprednisolone (Depo-Medrol, Pharmacia and Upjohn, Puurs, Belgium) intramuscularly shortly before surgery, and animals in the non-steroid group remained untreated.

Low-level laser therapy

Three wounds in each rat were irradiated daily using a commercially available InGaAlP diode laser (Maestro/CCM, Medicom Praha, Prague, Czech Republic; λ=670 nm, shape of beam oval, S 1 cm^2) to provide a total daily dose of 5 J/cm^2, and the fourth wound was not irradiated and served as a control. The three treated wounds were irradiated with power densities of 5, 15 and 40 mW/cm^2, respectively. The positions of the treated and control wounds were rotated within the groups. During wound treatment, the rats were restrained in a Plexiglas enclosure with an oval opening over the currently stimulated wound and with the other wounds protected from reflected laser light.

Histopathological evaluation

All animals were killed by ether inhalation 6 days after surgery (a period sufficient for assessment of the proliferation phase and re-epithelization of the wounds) [18]. Tissue specimens were processed routinely for light microscopy (fixation, dehydrating, embedding, sectioning, and staining with haematoxylin and eosin (H&E, basic staining) and van Gieson (VG, nonspecific collagen staining).

A semiquantitative method [18] was used to evaluate the following histological features: polymorphonuclear leucocytes, re-epithelization, fibroblasts, new vessels and collagen. Sections were evaluated in a blinded manner on a scale of 0–4 (Table 1).

Statistical analysis

For each evaluated parameter mean±SD values were calculated. The data obtained from the semiquantitative evaluation were compared using the nonparametric Kruskal-Wallis test. Significance was accepted at $p<0.05$.

Results

After surgery the animals remained healthy without clinical evidence of infection. The results of the histological investigation are summarized in Fig. 1.

Table 1 Semiquantitative histological evaluation of healing skin wounds

Scale	Re-epithelization stage	Polymorphonuclear leucocytes	Fibroblasts	New vessels	Collagen
0	Thickening of cut edges	Absent	Absent	Absent	Absent – granulation tissue
1	Migration of cells (<50%)	Mild – surrounding tissue	Mild – surrounding tissue	Mild – surrounding tissue	Minimal – granulation tissue
2	Migration of cells (≥50%)	Mild – granulation tissue	Mild – granulation tissue	Mild – granulation tissue	Mild – granulation tissue
3	Bridging the excision	Moderate – granulation tissue	Moderate – granulation tissue	Moderate – granulation tissue	Moderate – granulation tissue
4	Keratinization	Marked – granulation tissue	Marked – granulation tissue	Marked – granulation tissue	Marked – granulation tissue

Non-steroid group

The histological analysis in this group demonstrated completion of the inflammatory process in all wounds. Only treatment at the highest tested power density (40 mW/cm^2) was able to significantly reduce inflammation. Keratinocytes migrated beneath the scab, but did not completely bridge the excision. In wounds laser-treated at 15 mW/cm^2 significantly accelerated re-epithelization was seen. This period showed a typical histological picture of a proliferative phase with

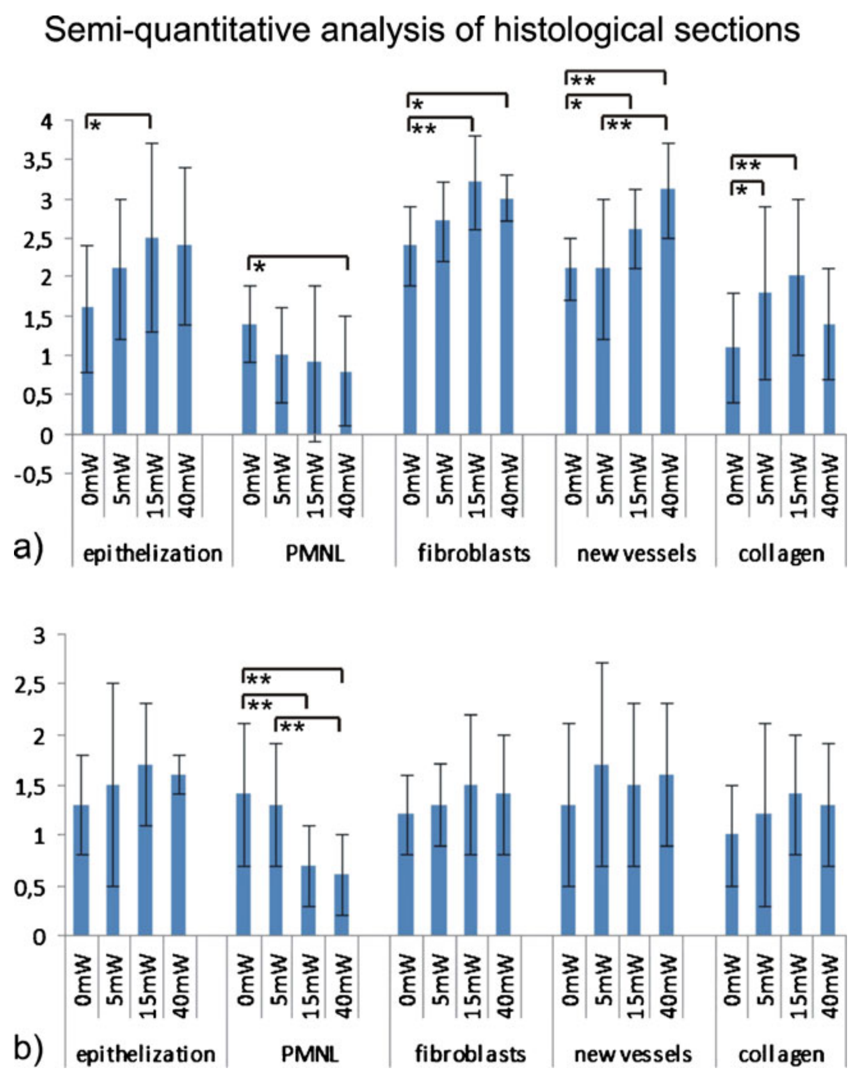

Fig. 1 Results of the semiquantitative histological evaluation of healing skin wounds following laser treatment: **a** non-steroid animals, **b** steroid-treated animals (*p<0.05, **p<0.01)

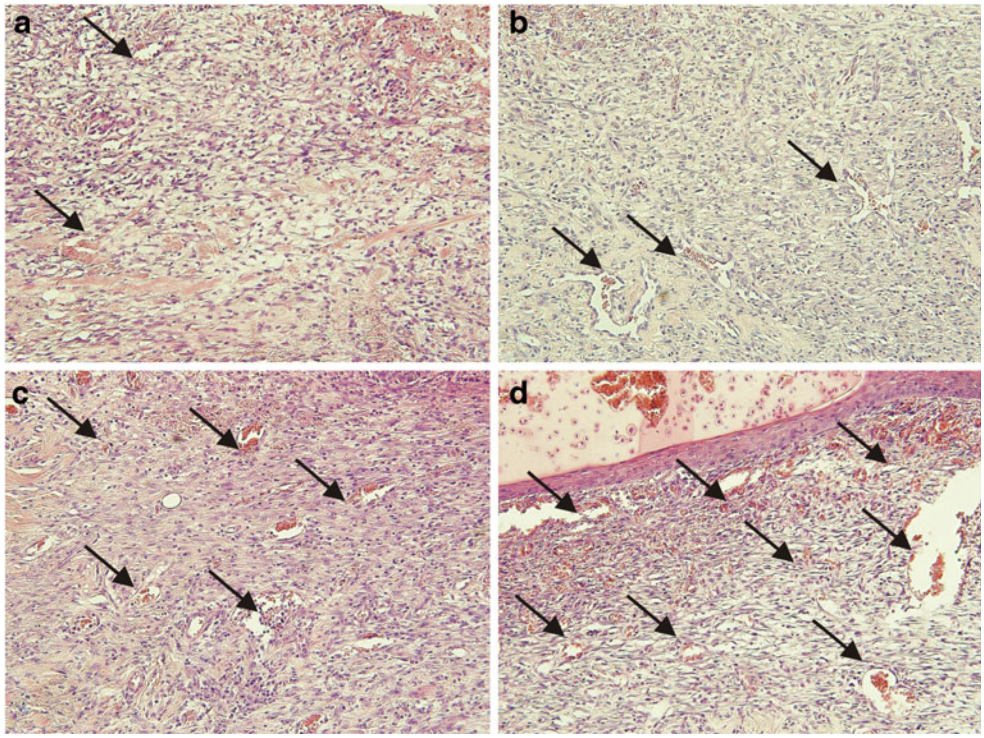

Fig. 2 Skin wounds 6 days after surgery in non-steroid rats: **a** control wound, **b** wound treated at 5 mW/cm^2, **c** wound treated at 15 mW/cm^2, **d** wound treated at 40 mW/cm^2. Newly formed vessels are seen to increase in a power density-dependent manner;(H&E staining, ×200)

expression of fibroblasts and new vessels in all wounds. Laser treatment at 15 and 40 mW/cm^2 significantly improved proliferation of fibroblasts and formation of new vessels in the granulation tissue of wounds (Figs. 1 and 2). Similarly, a greater amount of new collagen fibres was seen in wounds laser-treated at 5 and 15 mW/cm^2 (Fig. 3).

Steroid-treated group

Typical steroid-induced impairment of wound healing was seen in this group in which the inflammatory phase of healing was not complete. Nevertheless, LLLT at 15 and 40 mW/cm^2 was able to significantly reduce the amount of

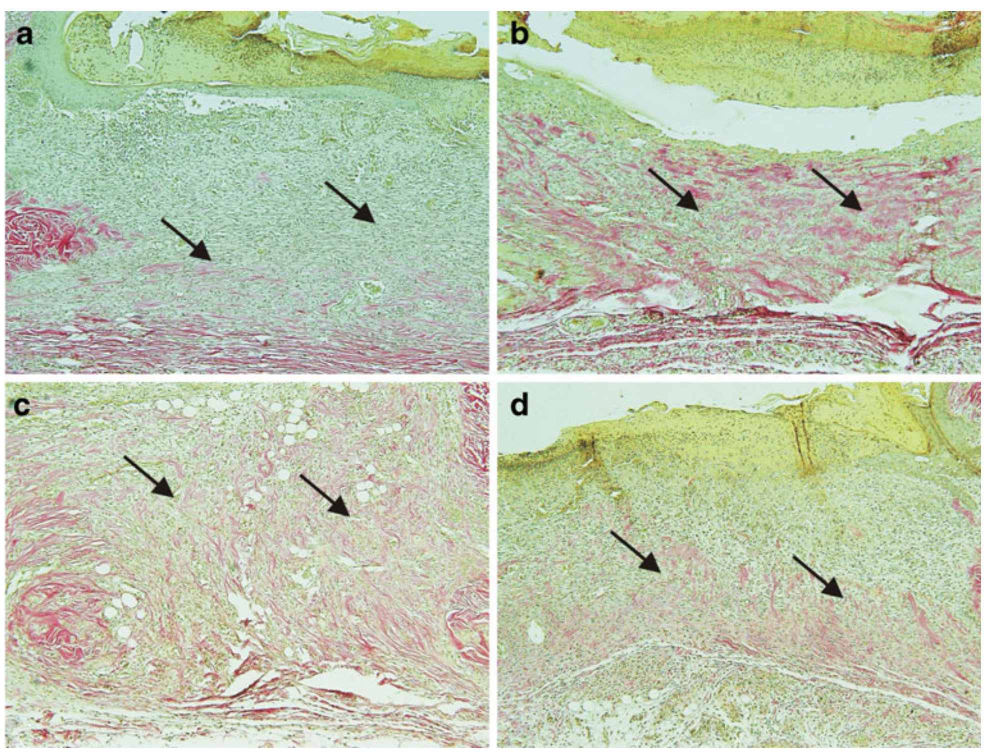

Fig. 3 Skin wounds 6 days after surgery in non-steroid rats: **a** control wound, **b** wound treated at 5 mW/cm^2, **c** wound treated at 15 mW/cm^2, **d** wound treated at 40 mW/cm^2. New collagen is seen in wounds treated at 5 and 15 mW/cm^2 (VG staining, ×200)

inflammatory cells at the injury site. In comparison to the wounds of non-steroid animals, the wounds of all steroid-treated animals showed markedly reduced granulation tissue. However, no significant differences in the evaluated histological parameters, including re-epithelization, presence of fibroblasts, new vessels and new collagen, between irradiated and nonirradiated wounds were observed.

Discussion

In general, it can be hypothesized that the mechanism of LLLT at the cellular level is based on an increase in oxidative metabolism in the mitochondria. This is supported by a study in which the effects of different wavelengths of LLLT (633, 670 and 820 nm) on redox changes in HeLa cells were compared. It was found that cytochrome *c* oxidase becomes more oxidized due to irradiation at each the wavelengths used [19]. However, comparing the effects of different wavelengths (670 and 685 nm) and intensities (2, 15 and 25 mW), achieving a total dose of 10 J/cm^2, LLLT was found to be more effective with the higher intensity combined with the shorter wavelength and with the lower intensity combined with the longer wavelength [13]. In addition, evaluating the effect of LLLT at 635 nm during the proliferative phase, re-epithelization and collagen synthesis were significantly accelerated in a power density-dependent manner [9]. Moreover, we have also found that red lasers are able to increase wound tensile strength. In contrast, to open wound healing, however, the 635 nm laser had a better effect on wound tensile strength at the higher tested power density and the 670 nm laser at the lower power density [20].

In general, the main difference between primary and open/secondary wound healing is in the amount of granulation tissue formed. Whereas a primary wound heals either without granulation tissue or with a minimal amount of granulation tissue, a secondary wound needs new tissue development which includes extensive granulation tissue formation [18]. Since lasers have a wavelength-dependent tissue penetration, the energy absorbed in each skin layer differs for each laser. Hence, depending on the absorbed doses of low-level laser radiation, it has been shown that cells might be stimulated either to proliferation [21], differentiation [22], collagen production [23] or apoptosis [24]. Therefore, for the use of LLLT in clinical practice both laser parameters and wound type must be taken into consideration.

As in our previous study, evaluating the effects of LLLT during the proliferative phase of healing showed that selected parameters were significantly accelerated only in non-steroid animals. In contrast to those rats, the steroid-treated rats showed less granulation tissue formation with no significant differences between stimulated and control wounds. This was shown in our previous study [18] as well as in the current investigation. In contrast to our results, it has been shown that LLLT at 904 nm and 33 J/cm^2 stimulates the proliferative phase of healing in both non-steroid and steroid-treated animals which received 2 mg/kg of dexamethasone daily [8, 25]. This appears to be the limit of the efficacy of LLLT. With the high corticosteroid dose used in the current and our previous investigations [9], low-level laser treatment does not improve proliferation, but does have an antiinflammatory effect.

In conclusion, our results extend and reinforce the theory of the positive effect of LLLT on wound healing under normal conditions. Moreover, our previous study and the current study have shown that LLLT at either 635 nm or at 670 nm does not significantly accelerate wound healing after corticosteroid treatment using the present methodology. Furthermore, the inverse relationship between power density and the laser effect as well as the discrepancy in the effect of irradiation at 670 nm on the healing of primary sutured and open wounds might be important for further clinical research.

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