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Equine wound healing: influence of low level laser therapy on an equine metacarpal wound healing model

Wundheilung beim Pferd: Untersuchungen zur Wirksamkeit der Low-Level-Laser-Therapie am Wundheilungsmodell (Mittelfußregion)

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Abstract

Objective: To evaluate the effects of low level laser therapy (LLLT) on healing of full thickness symmetrical skin wounds in horses. LLLT is a therapeutic modality using the application of light, usually a low power laser or light emitting diode in the power range of 1 mW to 12 W that, in practical terms, promotes tissue regeneration as well as reducing inflammation and pain.
Study design: Experimental study.
Animals: Healthy horses (n=8).
Methods: Full thickness, 2.5 cm square skin wounds were created in the mid-metacarpal region on one leg of eight normal horses. LLLT was used on limbs assigned to the experimental group and limbs assigned to the control group were allowed to heal without treatment. LLLT was administered using a line generated optical scanner with a dual diode laser system (model EML; Erchonia Laser Healthcare, McKinney, TX, USA) at a wavelength of 635 nm and an energy output of 17 mW per diode. Wound size was measured for an 80-day period post operatively. Eighty days after surgery incisional biopsies were examined histologically.
Results: Wounds treated with LLLT healed faster than the control wounds (p=0.01). Wounds treated with LLLT were completely epithelialized at day 80 after surgery. Control wounds were not epithelialized at postoperative day 80.
Conclusion: LLLT increased the rate of wound healing.

Keywords: wounds; wound healing; metacarpal; LLLT; epithelialization.

Zusammenfassung

Zielsetzung: Evaluation der Wirksamkeit der Low-Level-Laser-Therapie (LLLT) auf die Heilung symmetrischer Hautwunden (Vollhaut) bei Pferden. Bei der LLLT wird Licht, üblicherweise von Low-Power-Lasern oder LEDs im Leistungsbereich von 1 mW bis 12 W, appliziert, resultierend in einer verbesserten Geweberegeneration, Entzündungshemmung und Schmerzlinderung.
Studiendesign: Experimentelle Studie.
Tiere: Gesunde Pferde (n=8).
Methoden: Im Mittelfußbereich jeweils eines Beins von 8 gesunden Pferden wurden 2,5×2,5 cm² große Hautwunden (Vollhaut) chirurgisch erzeugt und entweder mittels LLLT behandelt (Experimentalgruppe) oder keiner speziellen Therapie unterzogen (Kontrollgruppe). Für die LLLT kam ein optischer Laserscanner der Fa. Erchonia Laser Healthcare (Modell EML, duales Laserdiodensystem; Wellenlänge: 635 nm, Leistung: 17 mW pro Laserdiode) zum Einsatz.

Die Wundgröße wurde post-operativ über einen Zeitraum von 80 Tagen vermessen. Mit Ende des Kontrollzeitraums, 80 Tage nach dem chirurgischen Eingriff, wurden Gewebeproben (Inzisionsbiopsie) entnommen und histologisch untersucht.
Ergebnisse: Die Hautwunden, die mit LLLT behandelt wurden, heilten schneller als die unbehandelten Wunden aus der Kontrollgruppe (p=0.01). 80 Tage nach dem chirurgischen Eingriff waren alle mittels LLLT behandelten Wunden vollständig epithelisiert. Dies war in der Kontrollgruppe nicht der Fall.
Zusammenfassung: Eine Behandlung mittels LLLT verbessert die Wundheilungsrate.

Schlüsselwörter: Wunden; Wundheilung; Mittelfußbereich; Low-Level-Laser-Therapie (LLLT); Epithelisierung.

1. Introduction

Low level laser therapy (LLLT) is a therapeutic modality using the application of light, usually a low power laser or light emitting diode in the power range of 1 mW to 12 W that in practical terms promotes tissue regeneration as well as reducing inflammation and pain. LLLT can be defined using several parameters that include power, wavelength, pulse rate and duration, total irradiation time, intensity (W/cm^2), and dose (J/cm^2). Classifications of lasers, designated by the American National Standards Institute, used for LLLT range from Class 2 through 4. Conceptually, LLLT is not considered a photothermal activity but in practice – using some Class 3B and 4 devices – photothermal activity (not including tissue ablation) may occur to a limited extent. The basic mechanism, as defined for LLLT, is considered photochemical or tissue activity through photobiomodulation or photobiostimulation [1, 2]. This study utilizes a Class 3B LLLT device to enhance wound healing in the equine wound model.

Wound healing in the distal limbs of horses is an aspect of veterinary medicine that has made few advances in spite of our expanding knowledge and technological progress. In fact, treating distal leg wounds (DLWs) and the ensuing exuberant granulation tissue remains one of the most frustrating clinical challenges [3]. There have been countless topical preparations and medications that have been applied to DLWs over the millennia and even in recent times, but few have actually been scientifically shown to possess significant clinical efficacy. There have been numerous studies comparing treatments such as corticosteroids [4], platelet rich plasma [5], occlusive dressings [6], and collagen gel [7], growth factors [8] and immobilization [9], but no treatment has been shown to have a statistically significant effect on the healing rate of full thickness excisional skin wounds. The goals of treating DLWs include minimizing exuberant granulation tissue and facilitating contraction and epithelialization. Minimizing scar tissue and adhesion of underlying deep structures are also important considerations [10].

LLLT has been shown to be beneficial in facilitating healing of slow or non-healing wounds in laboratory animals [11] and humans [12–15]. LLLT stimulates cellular physiologic processes [16]. Near-infrared light stimulates cytochrome c oxidase located within the mitochondrial respiratory chain [17–19]. This causes up-regulation of oxidative phosphorylation and increased production of adenosine triphosphate (ATP) [20, 21]. Increased ATP availability augments intracellular and extracellular signaling resulting in pain and edema reduction [22–24]. Efficacy of LLLT in wound healing, control of inflammation, and pain management has also been documented [25, 26]. The physiological effect of LLLT is one of direct stimulation of tissue without thermal vaporization. A visible light is emitted with a wavelength of 630–640 nm that easily passes through dermal tissue [23, 27]. Documented effects of LLLT include increased production of growth factors, cellular matrix, angiogenesis, and cytokine release [28, 29]. Other events critical to wound healing that occur between 630 and 640 nm include activation of fibroblasts and keratocytes, DNA replication, cellular proliferation, and angiogenesis [30–32]. This experiment attempted to document the effects of LLLT (635 nm wavelength red laser light) on the healing characteristics of surgically created identical and symmetrical DLWs in a controlled environment.

2. Materials and methods

2.1. Horses

Eight mature mares were studied. Age range was 5–12 years and weight range was 464–615 kg.

2.2. Surgical procedure and LLLT treatment

Treatment protocols of the animals in this project were approved by the Institutional Animal Care and Use Committee. Application of the low level ANSI Class 3B medical laser for this project was utilized in accordance with ANSI Z136.3 guideline "American National Standard for Safe Use of Lasers in Health Care" (2005).

After induction and maintenance of general anesthesia, DLWs were created on limbs of eight mature horses. A unilateral full thickness 2.5 cm square excisional skin wound was created on the dorsal mid-metacarpal region equidistant from the carpometacarpal and metacarpophalangeal joints of each horse. The wounds were created with a #10 scalpel blade after tracing the outline with a stencil (Figure 1). Hemostasis was obtained with electrosurgical coagulation and all legs were bandaged for five days after surgery. Post-operative analgesia was provided with flunixin meglumine (1.1 mg/kg at 12 h) for three days.

A randomized experimental design was used so that an equal number of right and left legs were used for both control and experimental data points. LLLT was applied to the experimental wounds every other day for 80 days. The laser beam target area was determined by manually maintaining the optical scanner approximately 3 cm from the wound area and irradiating a 4 cm^2 area (linear beam size) directly over the wound site (Figure 2). LLLT was administered using a line generated optical scanner with a dual diode laser system (model EML; Erchonia Laser Healthcare, McKinney, TX, USA) at a wavelength of 635 nm and an energy output of 17 mW per diode. Laser therapy was initiated and applied at the wound site for 5 min with pre-set pulsed laser therapy frequencies alternating from 28, 16, 12, to 4 Hz, with a 50% duty cycle from each diode, i.e., as the laser is a dual diode system one diode will pulse 28 and 16 Hz rotating frequencies every second and the other diode would pulse at 12 and 4 Hz.

Figure 1 2.5×2.5 cm^2 full-thickness skin wound.

Figure 2 LLLT of metacarpal wound using a dual-diode, line generated optical scanner (model EML; Erchonia Laser Healthcare, McKinney, TX, USA).

These energy parameters were established according to the manufacturer's specifications. Total energy delivered to the wound was 5.1 J/cm^2 per treatment.

In conjunction with direct wound treatment, irradiation/LLLT of the associated nerves proximal to the wound site (median/musculocutaneous nerves as they exited the brachial plexus) was also performed. The same optical scanner and dual diode laser system used for wound treatment was also employed for this aspect of the protocol. The scanner was held in contact or within 1 cm of the skin over the nerve trunks as they coursed distally to the area of the wound site. Laser beam size for this aspect of the LLLT was 1 cm^2. Pulsed laser delivery frequencies were set at alternating frequencies from 9, 16, 33, to 60 Hz. Total irradiation time was 3 min for total energy delivery to the "trigger point" of 1.6 J/cm^2 per treatment.

Wound size area was assessed digitally using ImageJ software (ImageJ 1.44p; National Institutes of Health, Bethesda, MD, USA). The wound size was calculated and wound healing curves were generated. Control curves were compared to experimental curves and a statistical analysis was obtained.

2.3. Statistical analysis

All statistical analyses were conducted with PC SAS Version 9.2 (SAS Institute, Cary, NC, USA). Analysis of variance procedures assuming a repeated measures model were utilized to ascertain the effects of treatment, time and the treatment by time interaction. An autoregressive analysis with time period 1 covariance structure was used to model the intra-horse variation over time. The analysis focused on wound data measured after day 26. The interaction of treatment by time was assessed, and if not significant, main effects of time and treatment evaluated. All tests were considered significant if $p<0.05$.

3. Results

3.1. Rate of wound healing

The time by treatment interaction was not significant ($p=0.9802$), which enabled us to evaluate the main effect of treatment (averaged over time). The effect of treatment averaged over time was significant (control mean=0.25 vs. treatment mean=0.15; $p=0.0134$). There was a steady decrease in wound size for both control and experimental wounds after day seven. The rate of healing was statistically different for the control and experimental (LLLT) wounds after day 17 (Figure 3). Experimental wounds were fully contracted and epithelialized by day 80 (Figure 4B). Control wounds were approximately 9% larger and not fully epithelialized (Figure 4A). The time for complete epithelialization of the control wounds was not determined because the study ended with the biopsy procedure. The fastest rate of decrease in wound size was between day 19 and day 46 for both control and experimental wounds. During this time period the slope of the wound healing lines was the steepest.

3.2. Histologic evaluation

The wound site dermis of both control and laser-treated horses was expanded and effaced by deposition of densely cellular and highly vascular granulation tissue. The granulation tissue was abruptly demarcated from the adjacent dermis at the margin of the wound. All control animals exhibited ulceration of the wound site with the exposed granulation bed covered by a thick serocellular crust comprised of an admixture of fibrin, serum protein and intact and degenerate neutrophils. The immediate underlying granulation tissue was heavily infiltrated by primarily neutrophilic inflammatory cells. The epithelium at the margins of the ulcer was markedly hyperplastic with formation of deep, irregular pegs. Some animals exhibited sliding of isolated nests of hyperplastic epithelial cells across the ulcer bed; however, these were always discontinuous. In contrast, all treated horses exhibited intact epithelial surfaces. The granulation bed was completely covered by evenly hyperplastic epithelium with orthokeratotic hyperkeratosis. Some animals exhibited hemorrhage within the granulation region, but this was always mild. Inflammation was minimal and restricted to few, scattered mononuclear cells within the granulation bed (Figure 5A,B).

4. Discussion

The rate of healing of non-treated wounds was consistent with previous reported data for horse wounds [10]. These results of this study are similar to a previously reported study investigating the use of extracorporeal shock wave therapy in which a similar model and wound measurement methodology was used [33]. The laser-treated wounds had a more advanced rate

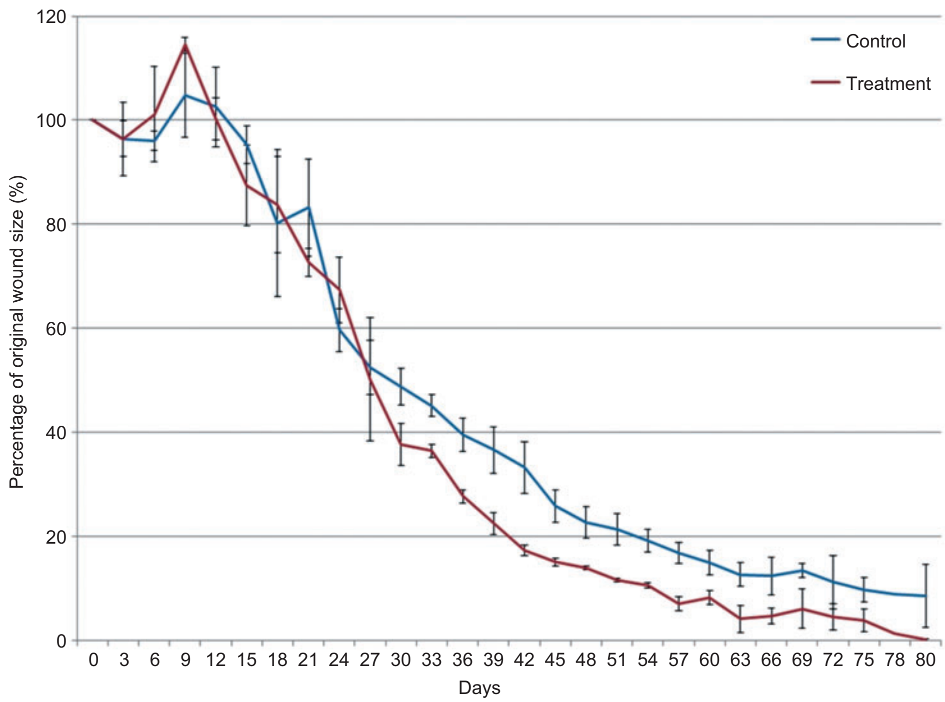

Figure 3 Final graph of mean percentage of wound size vs. time (days) for control and laser-treated (LLLT) wounds.

of healing than non-treated wounds in this study and previously reported healing rates [10]. Our clinical impression was that the laser-treated wounds healed primarily by wound contraction in the manner previously reported for pony wounds [10]. A significant clinical observation was the absence of exuberant granulation tissue in the laser-treated wounds. The wound margins of the laser-treated wounds contracted and epithelialized and the overall contour of the wounds

Figure 4 Appearance of (A) control and (B) laser-treated wounds at day 80.

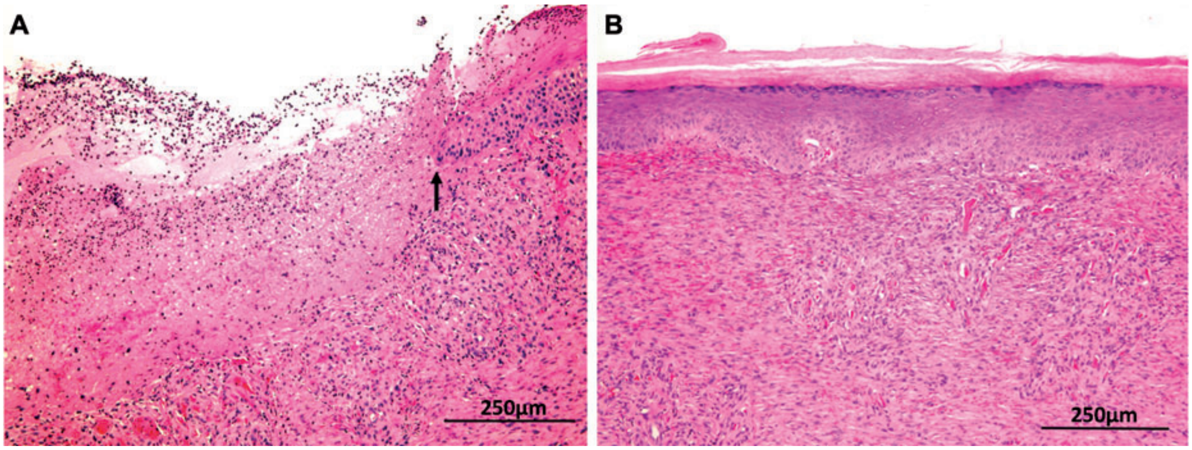

Figure 5 Horse, skin. Hematoxylin and eosin stains. (A) Control horse skin exhibited ulcers over the granulation bed at the wound site. The ulcers were covered by serocellular crusts with epithelial hyperplasia at the margins (arrow). The underlying granulation bed was inflamed (neutrophilic). (B) The granulation bed at the wound site from treated horses was completely covered by evenly hyperplastic epithelium with orthokeratotic hyperkeratosis. Inflammation was minimal and characterized by few, evenly scattered macrophages and lymphocytes within the granulation bed.

remained flat and parallel to the surrounding epithelium. This is potentially clinically relevant because control of exuberant granulation tissue is a pivotal factor in management of equine wounds located in the distal limb.

LLLT of the metacarpal wounds included direct wound treatment as specified, as well as LLLT of the associated nerves proximal to the wounds site. In this study, the LLLT device employed for direct wound treatment was also used for irradiation of the median/musculocutaneous nerves proximal to the wound site. Direct and nerve root irradiation as well as treatment of "trigger points," are frequently discussed in relation to complementary medicine techniques, namely "laser acupuncture". A complete discussion of that topic is beyond the scope of this paper. However, previous work has shown that both pain relief and enhanced wound healing can be initiated using direct and indirect LLLT effects on cellular activity. Direct cellular activation is postulated to lead to indirect activation of distant cells via secondary messengers (reactive oxygen species, lymphokines, cytokines, nitric oxide, and calcium) released by directly activated cells [12, 34, 35]. In this study, it was not possible to ascertain whether treatment of the nerves proximal to the wound site enhanced wound healing or alleviated potential discomfort at the wound site.

5. Conclusion

LLLT had a positive effect on healing rate and healing characteristics of DLWs in horses.

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