

Effect of infrared and red monochromatic light on equine wound healing

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Abstract

Background: Light-emitting diodes (LEDs) are commonly used for treating a variety of disorders in horses, including wounds. Despite its claim to shorten healing times, there is a lack of scientific documentation regarding its effects.

Objectives: To investigate if treatment with pulsating visible red light ($\lambda \approx 637$ nm) and near-infrared (NIR) light ($\lambda \approx 956$ nm) affects wound healing.

Study design: Randomised blinded controlled experimental study.

Methods: A circular skin wound ($\varnothing = 2$ cm) was created on each side of the neck in eight healthy horses. One randomly chosen wound received light treatment and the other served as an untreated control. Treatment duration was 4 minutes and 40 seconds (red light 95 seconds, 2.3 mW/cm²; NIR light 185 seconds, 6.4 mW/cm²) and was performed once daily on day 0-4, 7-11, 14-18 and 21-25. The wounds were photographed and evaluated using digital photoplanimetry on day 0, 1, 2, 3, 4, 7, 14, 21, 28 and 35. The degree of swelling was assessed with diagnostic ultrasound on the same days except the last recording was performed on day 36 instead of 35. Days to total healing was recorded. ANOVA was used for statistical analysis ($P < .05$).

Results: The wound area ($P = .2-.9$) and degree of swelling ($P = .2-1.0$) did not differ between treated and control groups on any day. There was a significant difference ($P = .03$) in healing time between control (49.0, 95% CI = 35.4-62.6 days) and treated wounds (51.8, 95% CI = 38.7-64.8 days).

Main limitations: The wounds were treated until day 25 and this study does not investigate the effect of a longer treatment period than 25 days.

Conclusions: The results of this study do not indicate any clinically relevant positive effect of pulsating visible red light and NIR light on the healing of experimental skin wounds in horses, compared with no treatment.

KEYWORDS

horse, swelling, low level light therapy, wound

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1 | INTRODUCTION

Irradiation with light-emitting diodes (LEDs), so called photobiomodulation, has shown promise for stimulating wound healing.¹ Treatment with LEDs is considered non-invasive, non-thermal and with few side effects. Unlike lasers, LEDs emit non-coherent light possibly allowing treatment of larger surfaces and superficial tissue such as cutaneous wounds.² Several mechanisms of action have been proposed, with the most accepted theory being that LEDs have an effect on the mitochondrial respiratory-chain enzyme cytochrome c oxidase (CCO). CCO acts as a chromophore and the absorption of light leads to a variety of intracellular changes including increased electron transfer which in turn increase adenosine triphosphate levels.³⁻⁵

In *in vitro* studies, visible red and near-infra red (NIR) light treatments have been shown to stimulate the proliferation of fibroblasts,⁶⁻⁸ endothelial cells⁹ and keratinocytes,¹⁰ cell types that are essential to wound healing. *In vivo*-studies, mainly in rats and mice, have investigated the effect on wound healing with varying results.¹¹ However, LED light treatments with the wavelengths 670, 720 and 880 nm increased wound healing rates in a diabetic mouse model.¹² Further, LED light (880 nm) suppressed the enzyme cyclooxygenase-2 and may thus have an anti-inflammatory effect in rats.¹³ Therefore, red and NIR LED light could also have the potential for treating oedema and inflammation. These effects have led to expectations for the clinical usefulness of low-level light treatment in horses. To date, no scientific documentation on the effect of LEDs on wound healing in horses has been published. Thus, the purpose of the present study was to investigate how a commercial LED device, marketed for treatment of wounds in horses by use of both pulsating visible red light ($\lambda \approx 637$ nm) and NIR light ($\lambda \approx 956$ nm), affects swelling and wound healing in healthy horses. The hypothesis was that LED light treatment of experimental wounds in horses will reduce swelling, decrease wound area and time to total healing, compared with no treatment.

2 | MATERIALS AND METHODS

2.1 | Horses

Eight adult Standardbred horses participated in the study (6 mares, 2 geldings; age 12 ± 5 years; weight 524 ± 47 kg). The horses were kept under identical housing conditions and turned out in a paddock during the daytime. The horses were deemed healthy after a general clinical examination. The horses were randomly allocated an identity by using numbers (1-8). The study was performed during March to April.

2.2 | Skin wound

Horses were sedated with a combination of 4 mg detomidine hydrochloride (Domosedan, Orion Pharma AB Animal Health), and 4 mg

of butorphanol tartrate (Butomidol, Salfarm Scandinavia AB) given intravenously. A 6 cm \times 6 cm rectangular area was shaved at a standardised location on each side of the neck. The areas were aseptically prepared. In the centre of the area, a subcutaneous injection of 2 mL 2% w/v mepivacaine hydrochloride without adrenaline (Carbocain, AstraZeneca) was administered for local anaesthesia. Thereafter, full thickness skin wounds were created, by the same surgeon with a custom made, 2 cm diameter circular punch (Ångström Laboratory, Uppsala University), in the centre of each shaved area.

All wounds were left unprotected for second intention healing except on day 3, 10 and 11 due to wet weather and high risk of heavy contamination. On these days the wounds were covered with a non-adhesive sterile primary dressing that was attached to the skin with adhesive foam dressing. Each wound was uniformly cleansed with sterile 0.9% w/v sodium chloride (Natriumklorid Fresenius Kabi, Fresenius Kabi) one time daily from day 1 to 15, thereafter the wounds were only cleansed when deemed necessary for proper wound evaluation. An equal volume of saline was used on each wound. Scabs were removed if needed, to assess the wound and in these instances, scab removal was performed bilaterally.

2.3 | Treatment

Treatment location was randomly assigned and four horses received treatment on the left side and four horses on the right side. Wounds were treated once daily on day 0-4, 7-11, 14-18 and 21-25, and the treatment was performed by the same two people. Treatment was conducted with a handheld device (BCD 650 Animal, Biolight AB) that was held approximately 1 cm from the wound with the help of spacers and the light beam covered the entire wound surface. Treatment consisted of a pre-set programme with a duration of 4 minutes and 40 seconds. Red light was emitted for 95 seconds and NIR light for 185 seconds. Before commencing the study, the emitted wavelengths were measured with a spectrometer [CCS200, Thorlabs] and showed that the device emitted light in the red ($\lambda \approx 637$ nm) and NIR ($\lambda \approx 956$ nm) part of the spectrum. The irradiance was measured using a power meter [PM310D, Thorlabs] and a thermal power sensor [S310C, Thorlabs]. The irradiance for red light was measured to 2.3 mW/cm² and for NIR light to 6.4 mW/cm². Total energy over the entire length of the program was calculated to 0.2 J/cm² respectively 1.2 J/cm² for each wavelength.

2.4 | Wound evaluation

The horses were checked daily for general status, fever and signs of wound infection. The horses' manes were braided during the entire study period. Horses showing clinical signs of infection such as severe swelling, discharge or pain were treated adequately and excluded from the study.

Evaluation for swelling was performed on day 0-4, 7, 14, 21, 28 and 36 with a diagnostic ultrasound (LOGIQ e, GE Healthcare) by a

radiologist blinded to experimental group assignment. A linear probe (L4-12t-RS, GE Healthcare) with the setting to 2-3 cm and sterile saline as a coupling medium was used. On day 0, a baseline evaluation was performed, before the wounds were created, in the centre of the shaved area. On the remaining days, four locations in close contact with the borders of the wound were evaluated on the cranial, dorsal, caudal and ventral aspects of the wound.

The images were analysed in a software program (Centricity RA 600, GE Healthcare). The distance between the cutis and the adjoining muscle fascia was measured (depth of swelling), one distance in the centre of each image and one distance where the swelling was visually assessed as the maximum depth (Figure 1).

All wounds were photographed daily using the same digital camera (Canon EOS 550D, Canon) in a standardised manner. A ruler marked with code number and date was held to the skin. The photographs from day 0, 1, 2, 3, 4, 7, 14, 21, 28 and 35 were selected for analysis. Wound area was calculated using the image processing software Fiji (ImageJ, Version 1.51n).¹⁴ The wound edges were manually traced and related to a specific distance on the scale. All measurements were taken by the same blinded observer.

All wounds were visually examined daily to assess whether complete healing had occurred, by a person blinded to the experimental group assignment. The wounds were considered healed when an epithelial layer covered the entire wound surface.

2.5 | Data analysis

Wound area measured with digital photoplanimetry and days to complete healing were used for statistical analysis. A calculation of the sample size needed to detect an average mean difference of 0.25 mm for the maximum diameter of the wound at day 2 (SD 0.2131; power of 0.8), using a matched pair *t* test, resulted in a suggested sample size of $n = 8$. Normality was checked with diagnostic plots. Data are expressed as mean \pm SD. Wound area and swelling were analysed as repeated measures with standard mixed procedure in SAS (SAS Institute Inc.), where the model included the fixed effects

of treatment, examination day, the interaction between treatment and examination day, and the random effect of horse. Time to complete healing was analysed with paired *t* test. Significance level was set to $P < .05$.

3 | RESULTS

Wound areas were mildly swollen and slightly sensitive to palpation during the initial days after wound creation. None of the wounds showed clinical signs of infection such as severe swelling, discharge or pain. Thus, no horses were excluded from the study. The data included were from eight horses and 576 observations. There were no missing data. The normal approximation was good.

The mean values in cm for the two measured areas of swelling (maximum swelling and image centre) are presented in Figure 2. The result from the day 0 baseline evaluation was 0.37 ± 0.13 cm (maximal) and 0.30 ± 0.10 cm (centre). No significant differences were detected, on any of the evaluation days, between treated and control wounds (depth measured at image centre $P = .2$ -1.0; maximal depth $P = .6$ -1.0). The difference in wound area between treated and control wounds, as measured by digital photoplanimetry, was not significantly different on any of the evaluation days ($P = .2$ - $.9$). Wound area over time is illustrated in Figure 3.

Time to complete healing was significantly shorter for untreated control wounds (49.0 [95% CI = 35.4-62.6] days) compared with treated wounds (51.8 [95% CI = 38.7-64.8] days) ($P = .03$). Time to complete healing for all wounds are shown in Table 1.

4 | DISCUSSION

The results of this randomised blinded study show no significant differences in the degree of swelling nor in wound area between wounds treated with LEDS (pulsating visible red light and NIR light) and those not treated. There was a significantly longer time to complete healing for the treated wounds. However, the difference was

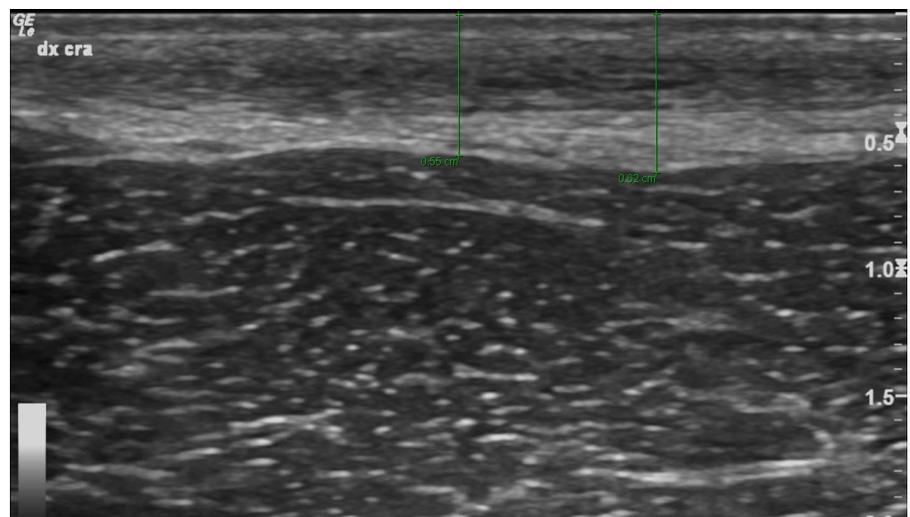


FIGURE 1 Example of measurements of swelling on an ultrasonographic image (centre of image = 0.55 cm; maximal distance = 0.62 cm)

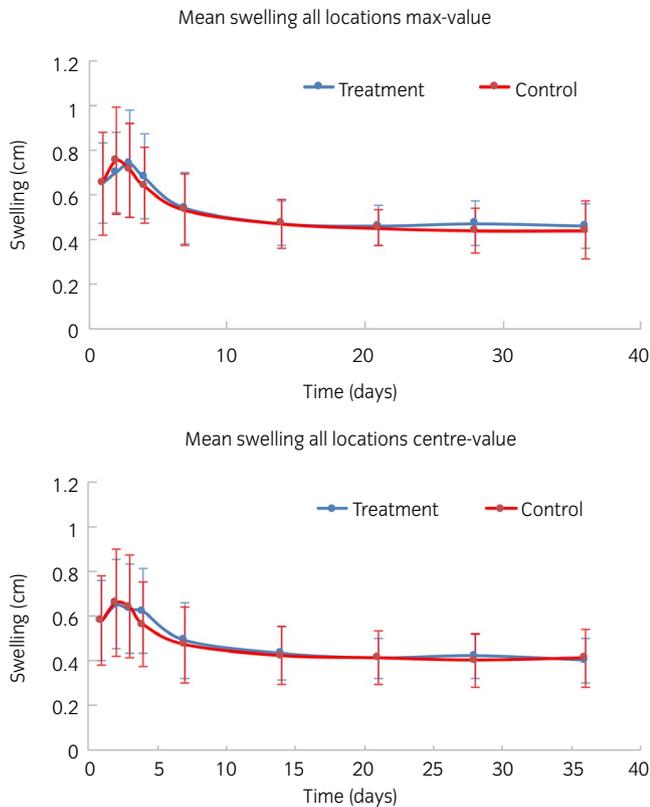


FIGURE 2 Mean values (in cm) for swelling at all aspects (cranial, ventral, dorsal, caudal) of the wounds for day 1-36, measured on a diagnostic ultrasound image. Max = maximum distance and centre = distance measured in the centre of the swelling

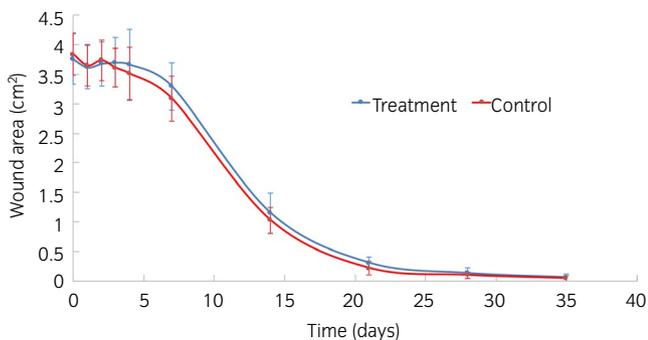


FIGURE 3 Mean wound area (cm²) over time in treated and control wounds, measured by digital photoplanimetry. Error bars represents ± 1 SD

small and probably of limited clinical relevance. Since there is a lack of research on LEDs and wound healing in horses, the results were also compared with studies on low-level light therapy performed with lasers instead of LEDs. The main differences between standard LED light and laser is that the latter is without phase-shifts (coherence), with uniform wavelength and is highly parallel (collimation), properties lacking in standard LED-lights. In published studies, differences in these properties, and also in irradiance (W/m²), irradiation dose and treatment protocols can make it difficult to compare results.¹⁵ Our results are in accordance with most other studies

TABLE 1 Time in days to complete healing for all wounds

Horse no.	Time to complete healing (d)	
	Treated	Control
1	36	31
2	76	70
3	39	39
4	69	70
5	45	44
6	36	31
7	77	76
8	36	31
Mean	51.75*	49*
Median	42	41.5
SD	18.8	19.7

* $P = .03$.

on low-level light treatment of wounds in horses.¹⁶⁻¹⁸ However, one non-blinded study on laser treatment in an equine metacarpal wound healing model recorded a positive effect in the late stages of healing.¹⁹ The time for secondary healing of similar sized untreated wounds differs between studies, ranging between 25 ± 3.5 days for wounds on ponies²⁰ to 44.0 ± 5.4 days for wounds on the body of horses²¹ and 80 days for wounds on horse limbs,¹⁹ indicating that the healing time described in the current study was similar to that previously reported.

Some of the treatment parameters (type of diodes, wavelengths, pulsation frequency, dose) were pre-set by the LED device. The treatment period was chosen after recommendations found in the available scientific literature as well as recommendations from equine rehabilitation therapists.^{22,23} It is possible that another protocol would have had a different effect; however, the selected protocol was the one that was predicted to have a positive effect based on existing knowledge. The wounds were treated until day 25 and this study does not investigate the effect of a longer treatment period than 25 days, which can be regarded as a limiting factor. The selection of wound location on the body was based on the expectation that wounds in this location heal faster and with less exuberant granulation tissue formation than wounds on the limb.^{20,21,24} Further, the use of one wound on each side of the horse's neck reduced the magnitude of inter-individual variability among the animals. It is claimed that the effect of photobiostimulation is dependent on the physiological condition of the cells treated, that is, that healthy cells should respond differently than sick cells.²⁵ In the current study, the wounds were experimentally created and may not behave exactly like more complex naturally occurring injury; however, the wounds were exposed to the same external environment as other wounds caused by other types of trauma.

The effect on swelling and wound area was assessed by objective outcome tools. The degree of swelling was assessed by use of diagnostic ultrasound and wound area by digital photoplanimetry,

techniques routinely used in wounds healing studies.^{18,21,26–28} The digital photoplanimetry needs not to interfere with wound healing, but when the wounds are covered with scabs, there is an element of uncertainty regarding the exact borders of the wound. The same problem appears with the visual examination estimating complete healed. In order to reduce this recording error, remaining scabs were removed, a procedure that may have interfered with the healing process. However, to avoid the influence of scab removal and cleansing on the study results, if one wound needed scab removal or cleansing, the contralateral wound was also cleaned.

The results of this randomised blinded study do not indicate any positive effect of pulsating visible red light and NIR light on the degree of swelling, wound area nor time to complete healing of 2 cm experimental neck skin wounds in horses, compared with no treatment. In fact, in our study, the LED light-treated wounds took longer to heal completely than control wounds.

ETHICAL ANIMAL RESEARCH

This study was approved by Uppsala Animal Experiment Ethics Board (C67/16).

OWNER INFORMED CONSENT

Not applicable.

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CONFLICT OF INTERESTS

No competing interests have been declared.

AUTHOR CONTRIBUTIONS

P. Michanek was the principal author and contributed to study design, data collection and analysis, and manuscript preparation. T. Toth contributed to study design and preparing of wounds and revising the manuscript. E. Bergström contributed to data collection. H. Treffenberg Pettersson contributed to study design, ultrasound examinations, and revising the manuscript. A. Bergh was the senior author and contributed to overall study design, project coordination, data collection, data analysis, and revising the manuscript. All authors gave their final approval of the manuscript.

DATA ACCESSIBILITY STATEMENT

The data that support the findings of this study are available from the corresponding author upon reasonable request.

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Equine Wound Management

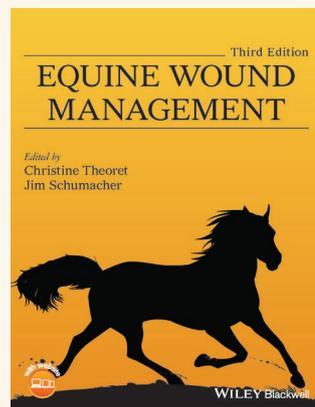
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