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ORIGINAL ARTICLE

Effects of weekly LED therapy at 625 nm on the treatment of chronic lower ulcers

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Abstract The aim of this study was to evaluate the effects of LED therapy associated with compression therapy on chronic venous leg ulcers (CVUs). The study included CVU patients (n=17, eight men) who were eligible for Unna's boot treatment. Ulcers were treated on a weekly basis with either LED therapy (625 nm, 4 J/cm^2) and an Unna's boot (LED ulcers, n=14) or a placebo treatment and an Unna's boot (control ulcers, n=14). The total surface area of the ulcers, the relative ulcer area, and the healing rate were recorded over a period of 30 weeks. Ulcer exudates were collected for determination of local tumor necrosis factor alpha (TNF-α) levels. The total area was not significantly different between the LED and control ulcers (P>0.05, Mann-Whitney U test) throughout the study. However, the relative area indicated that non-healing treatment resistant ulcers (n=6) persisted in the control group after 19 weeks (P<0.05, Mann-Whitney U Test). No differences in median healing rate were observed (hazard ratio 0.89, CI 95 %: 0.40–1.98) between LED (15 weeks) and control ulcers (19.5 weeks). No differences in TNF-α levels were detected (P>0.05, Mann-Whitney U test). The results suggest that LED therapy improved the effectiveness of the Unna's boot since no refractory ulcer was observed in the LED group after 19 weeks. However, LED therapy did not alter the local secretion of TNF-α nor accelerate wound healing.

Keywords Wound healing · Phototherapy · Inflammation · Exudates

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Introduction

Chronic venous ulcers (CVUs) are a complication of chronic venous disease and affect up to 3 % of the population over 60 years old [1, 2]. The high incidence and prevalence of CVUs have a great impact on public health programs, due to the high associated costs and long-term treatments [2, 3]. CVUs are associated with a poor quality of life and loss of productivity due to pain, impaired mobility, social isolation, and depression [4]. CVUs affect the legs and are commonly located over bone prominences, such as the medial gaiter area [2]. Several therapeutic approaches have been proposed, but the gold standard treatment is compressive therapy, associated or not with adjuvant interventions [2, 5, 6].

The most common etiological factor for CVUs is venous incompetence associated with venous hypertension [1, 2]. Venous incompetence causes blood stasis in the lower limbs, the accumulation of metabolites and edema in vessels and surrounding tissues, and capillary damage in addition to triggering inflammatory reactions [2]. Indeed, increased local levels of inflammatory mediators were detected in ulcer fluids

and debris from CVUs, and this was negatively correlated with healing time [7, 8]. A high expression of the inflammatory mediator tumor necrosis factor alpha (TNF-α) is associated with the impairment of wound healing, suggesting that targeting TNF-α may improve tissue repair [9–11]. In experimental models, blocking TNF-α using anti-TNF-α antibodies accelerated wound healing in a model of chronic ulcers using mice [9]. Another study demonstrated that applying antibodies topically against TNF-α could improve the healing rate in chronic ulcers that were resistant to conventional treatments [10]. However, none of the conventional compression treatments have direct anti-inflammatory effects.

Phototherapy employing laser or light-emitting diodes (LED) has been suggested for improving wound healing, due to its stimulatory effects on fibroblast proliferation, matrix synthesis, angiogenesis, and down-modulation of inflammatory reactions. It has been suggested that phototherapy with light wavelengths ranging from the red to near-infrared spectrum (600 to 980 nm) may accelerate wound healing in CVU patients [12, 13]. Phototherapy can also decrease the tissue expression of TNF-α [14–17], decreasing local inflammatory reactions in experimental and clinical studies. Phototherapy also promotes angiogenesis [18] and stimulates fibroblast proliferation [19–21] improving connective tissue repair and wound healing in experimental models. However, it is not clear if LED therapy is able to blunt local TNF-α production and accelerate wound healing in CVU patients treated with compression therapy. Few clinical trials have investigated the effect of phototherapy as an adjuvant therapy for CVUs, and their results to date are unclear [5].

The aim of this study was to evaluate the effects of phototherapy associated with standard compression therapy (Unna's boot) on the treatment of patients presenting CVUs. We investigated the healing area, healing time, and TNF-α levels in ulcer fluid in order to provide evidence of improved tissue repair when applying phototherapy as a coadjuvant treatment in CVU patients.

Methods

Subjects

The study was a randomized blinded clinical trial to evaluate the effects of LED therapy associated with the Unna's boot treatment in patients presenting chronic venous leg ulcers. Patients with diagnosed venous ulcers were enrolled from a Public Health Institution (Consórcio Intermunicipal de Saúde do Médio Paranapanema—CISMEPAR, Londrina-Brazil) from August 2010 to August 2012. The study was approved by the Ethics in Humans Research Committee of the Universidade Estadual de Londrina, and all patients were informed and signed an informed consent prior to participation in the study.

The inclusion criteria was the presence of active chronic leg ulcers with venous origin, classified as class C_6 by clinical manifestations, etiologic factors, anatomic distribution of the disease, pathophysiological findings (CEAP) scale. The ankle-brachial index <0.9 was also used to certify the venous etiology of ulcers. Exclusion criteria included non-venous ulcers, history of thrombosis, diabetes, and use of corticosteroids. All patients were diagnosed and accompanied by a vascular surgeon. A nursing team performed the cleaning of the ulcers and applied the Unna's boot weekly until either the ulcers healed or the patient dropped out of the study (infections or intolerance to treatment). LED treatment and ulcer exudates sampling were performed by a trained physiotherapist. Image records and analysis and and TNF-α quantification were performed by independent researchers blinded for sample allocation.

Experimental design

Patients with single or multiple ulcers on a single leg were randomly allocated into either phototherapy (LED ulcers, n=14) or placebo (control ulcers, n=14) treatment groups. Once a patient had been allocated into an experimental group, all ulcers on the affected leg received the same treatment. In patients presenting ulcers on both legs, the ulcers on the right leg were treated with LED therapy (LED ulcers) and the left leg ulcers were allocated to the control ulcers.

All ulcers were treated weekly with an Unna's boot. After the boots had been removed and the ulcers cleaned, the LED or placebo treatments were applied. In the control ulcers, the LED equipment was placed in position, but it was turned off (placebo treatment). During the LED therapy or placebo intervention, patients were laid in the supine position and wore an elastic blindfold covering their eyes. After LED irradiation or placebo intervention, a digital image was recorded and ulcer exudates were sampled.

LED or placebo treatments were applied until either the ulcers healed or for a period of up to 30 weeks. In the case of the ulcer not healing after 30 sessions, the LED or placebo treatments were interrupted and patients were treated exclusively with an Unna's boot.

Unna's boot treatment

The Unna's boot is an inelastic compression therapy composed of a multilayer zinc oxide-impregnated bandage. Unna's boot is the standard treatment employed by the Public Health Facilities in Londrina and was applied in an ambulatory environment by a nursing team. After the weekly removal of the boot, the ulcers were cleaned with sterile saline solution

and necrotic tissue, fibrin, and sphacelo were gently removed before phototherapy and sampling. After sampling, a new Unna's boot was applied. The procedures were repeated weekly until the ulcers healed.

LED therapy

Red low-level phototherapy was applied to ulcers using a prototype device built in the Laboratory of Optics and Optic Electronics at the Universidade Estadual de Londrina. A red wavelength probe contains seven 625-nm wavelength light-emitting diodes (bandwidth of 5 nm), a spot area of 1 cm^2, and an optical power output of 25 mW. The radiation was applied for 2 min and 40 s, administering 4 J/cm^2 of energy density, to an area of 1 cm^2. Total energy of 4 J per point was applied onto ulcers with an area <1 cm^2. In ulcers with an area >1 cm^2, five points of application were used: central, medial, lateral, distal, and proximal points (Fig. 1), with 4 J at each point, totaling 20 J of energy. The LED probe was positioned 2 cm above and perpendicular to the surface of the ulcer during irradiation.

Ulcer area

Ulcer areas were recorded weekly with a digital camera (Sony DSC-H5, 12 megapixels). A standard marker was positioned on the surface of the skin around the ulcers for standardization of captured images and data analysis. Photographs were analyzed using ImageJ® software (US National Institutes of Health, Bethesda, MD). A blinded investigator manually delimitated ulcer areas, as demonstrated in Fig. 2, to calculate the ulcer area. The total area was expressed in square centimeters. The relative area was expressed as the percentage of the remaining ulcer area in relation to the initial area ((total area in the evaluated week/total area in the first week) ×100) and expressed as percentage area (%).

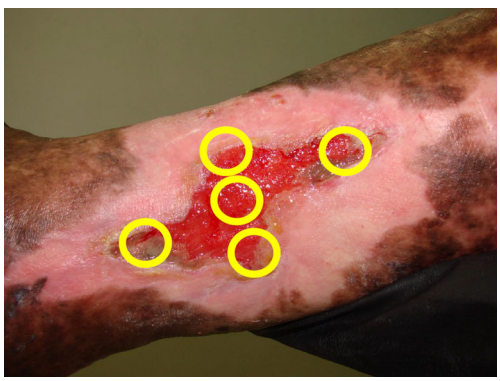

Fig. 1 Phototherapy points on a larger LED-irradiated ulcer

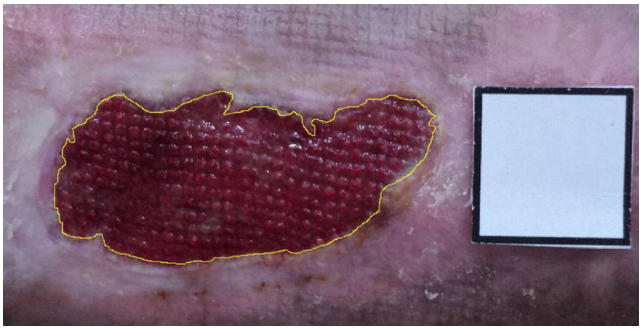

Fig. 2 Venous ulcer area recording. A digital image was transferred to the image software, and the ulcer area was manually delimitated (*yellow line*). A square adhesive marker (2×2 cm, *white with black borders*) was placed on the skin to the right hand side and used for standardization of color (pixels) and size of image

TNF-α detection in ulcer exudates

Patients were laid down in the supine position, and exudate samples were collected from the distal border of ulcers. Approximately 3 mm of the tip of sterile endodontic absorbent papers (n.20, Dentsply, York, USA) were placed on the border of the ulcer, on the connective tissue surface, and fluid was absorbed for 1 min. The absorbent paper was immediately placed in 0.5 ml of cooled sterile phosphate-buffered saline (PBS, pH 7.2) containing 50 mM phenylmethylsulfonyl fluoride and stored at −20 °C until use.

Samples were centrifuged at 20,000 rpm, at 4 °C, for 20 min, and TNF-α concentration was determined using an immunoenzymatic assay (OptEIA™ - Human TNF ELISA Kit II, Becton Dickinson Biosciences, Franklin Lakes, EUA) according to the manufacturer's instructions. Analysis was carried out in duplicate, and TNF-α levels were expressed as picograms per milliliter.

Statistical analysis

Normality distribution was determined using the Kolmogorov-Smirnov test. Data with parametric distribution were analyzed using the Student *t* test. Non-parametric data were compared using the Mann-Whitney *U* test. The hazard ratio was used to compare the median survival curve of ulcer healing in LED and control ulcers. Pearson product-moment correlation test was performed to detect correlations between ulcer area and healing time. Differences were considered significant if $P<0.05$.

Results

Seventeen patients were selected, of whom 15 (eight men) completed the follow-up. One patient was excluded due to topical hypersensitivity to the Unna's boot, and another did

not complete the treatment. All ulcers on the same leg were allocated to the same treatment, receiving LED therapy (LED group) or the placebo treatment (control group). Patients with unilateral ulcers were randomly distributed to the LED group (n=7 ulcers, three men and three women, mean age 58.03±17.83 years old) or the control group (n=5 ulcers, three men and two women, mean age 54.8±8.58 years old). The mean age did not differ between patients presenting unilateral ulcers (P>0.05, Student t test). Four patients (n=15 ulcers, two men and two women, mean age 67.5±11.38 years old) presented bilateral ulcers, and their lesions were allocated to the LED (right leg n=7 ulcer) and control (left leg n=9 ulcer) groups. There was no significant difference in mean time of evolution of ulcers between LED (n=14, 8.1±9.8 years) and control ulcers (n=14, 11.8±9.5 years, P>0.05).

Mean ulcers areas were not significantly different between the LED (n=14, 10.90±9.98 cm^2) and control (n=14, 9.91±6.14 cm^2) groups (P>0.05, Student's t test) before treatment. Only one ulcer in LED group (0.81 cm^2) and one in control (1.06 cm^2) were considered small and received only a single irradiation point (4 J). Six ulcers in LED group (mean area 4.2±2.5 cm^2, ranging from 2.2 to 9.2 cm^2) and control group (mean area 5.5±2.5 cm^2, ranging from 1.9 to 3.3 cm^2) were considered medium sized. Seven large ulcers were distributed in LED group (mean area 19.9±9.1 cm^2, ranging from 11.3 to 33.2 cm^2) and control group (mean area 17.7±8.7 cm^2, ranging from 10.2 to 34.1 cm^2).

From the first to the 25th week of treatment, there was no significant difference in the total area between the groups (Fig. 3a) (P>0.05, Mann-Whitney U test). However, the relative area of the ulcers expanded in the control group (n=8) after the 19th week (Fig. 3b). From week 19 to 25, the control group presented a median relative area ranging from 54.7 % (n=8, quartile 8 to 95.1 %, week 19) to 112.5 % (n=4, quartile 18.7 to 417 %, week 25) in relation to the initial ulcer area. The LED group presented a median relative area ranging from 13.5 % (n=6, quartile 2.9 to 40.4 %, week 19) to 9.8 % (n=4, 9.8 to 31.2 %, week 25). In fact, the LED group also presented non-healing ulcers (n=6) after the 19th week, but no increases in the total or relative ulcer area. Considering that larger ulcer areas may have a poor healing prognosis, we compared the initial area of non-healing ulcers (at 19th week) in LED group and control group. No differences in initial areas of non-healing ulcers of LED (n=6, median 8.0, quartile 2.6 to 24.2 cm^2) and control groups (n=6, median 8.1, quartile 4.4 to 27.4 cm^2) were detected (P>0.05). After the 25th week, three ulcers in each group had not healed, so statistical analysis was not continued. Three ulcers in the control group did not heal within 30 weeks. LED therapy did not accelerate the healing rate of ulcers during the 30-week period (Fig. 4). Median survival was not significantly different in the LED (15 weeks) and control (19.5 weeks) groups (Fig. 4). A weak correlation between initial ulcer areas and time to heal were observed in LED (r^2=0.30, P=0.29) and control (r^2=0.34, P=0.23) groups.

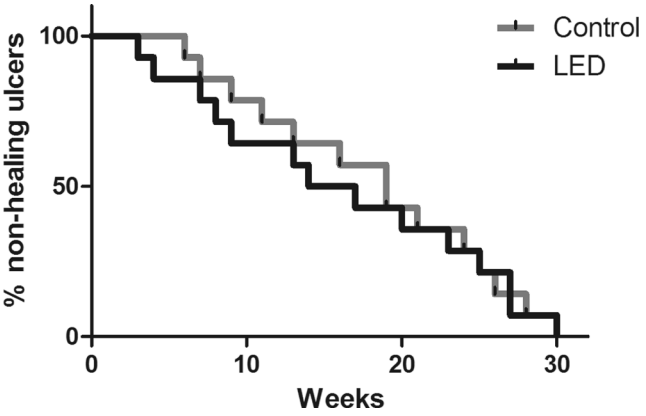

Fig. 4 Percentage of healed ulcers from the first to the 30th week of treatment in the LED and control groups. Hazard ratio 0.89 (confidence interval 95 % 0.40–1.98)

The initial TNF-α levels were 6.54±4.56 pg/ml (median=6.7, 25 to 75 % quartiles 2.3 to 10.6) in the LED ulcers and 4.88±4.22 pg/ml (median=2.87, 25 to 75 % quartiles 1.94 to 7.14) in the control ulcers, without statistical difference between the groups (P>0.05, Mann-Whitney U test)

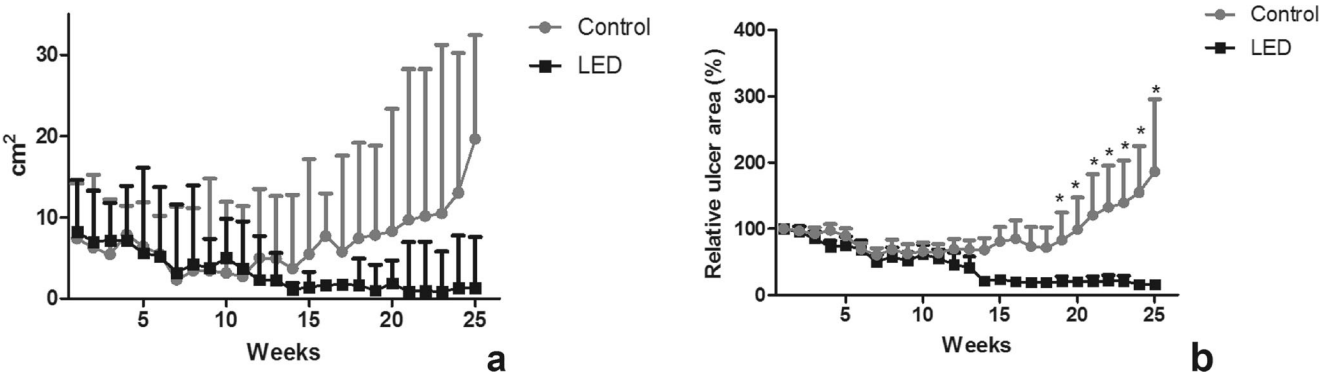

Fig. 3 Total area (**a**) and relative area (**b**) of ulcers submitted to LED therapy (*LED*) or the placebo (*Control*) treatment. Data are expressed in median and upper quartile (75 %). *P<0.05 in relation to the LED group, Mann-Whitney U test

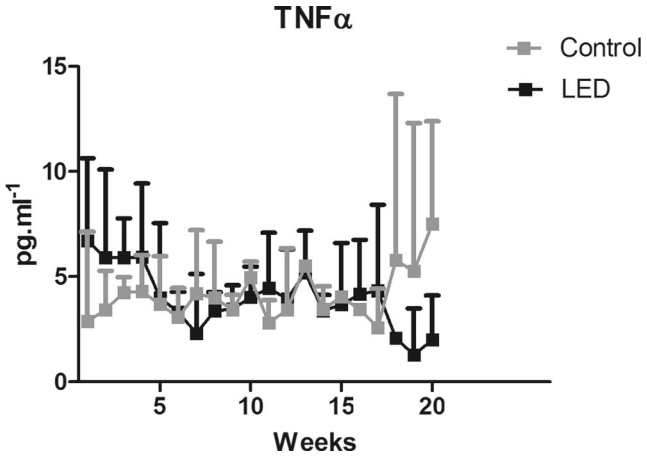

Fig. 5 Levels of TNF-α in ulcer exudates from the LED (*LED*) and placebo (*Control*) treatment groups. Data expressed as median and 75 % upper quartile

(Fig. 5). No significant differences in TNF-α levels were detected between the groups ($P>0.05$, Mann-Whitney U test) throughout the study. After 20 weeks, TNF-α levels were undetectable in LED ulcers.

Discussion

The main finding of the present study was that LED therapy seems to be effective in preventing the evolution of ulcers in the long-term treatment of CVU patients. Although phototherapy did not improve the healing rate, irradiated ulcers did not expand in area after the 20th week of treatment whereas remaining non-irradiated ulcers increased in relative area. This is an interesting issue in CVU treatment, as some patients are refractory to conventional compressive therapy, presenting non-healing ulcers over a longer period of time (more than 24 months) despite appropriate treatment [22, 23]. Avoidance of ulcer expansion in the LED group seemed not to be related to modulation of local levels of the main inflammatory cytokine TNF-α.

The standard treatment for CVUs is compression therapy, using compressive bandages [2, 1, 5, 6]. Compression therapy may be applied using a multilayer elastic bandage, inelastic bandage (Unna's boot), stocking, or intermittent pneumatic compression [24, 2, 5, 25]. The aim of compression therapies is to reduce local edema and improve the venous reflux [5, 2, 25]. The Unna's boot is a non-elastic bandage impregnated with zinc oxide. The topical treatment of ulcers with zinc has been suggested as improving wound healing due to its antioxidant and antimicrobial properties, besides stimulating debridement of connective tissue and epithelial proliferation [26]. However, an ideal approach to CVU treatment should also control local inflammation, stimulate tissue repair, and favor oxygenation of tissues [27]. Phototherapy may be a suitable adjuvant therapy to conventional compression treatment since it has anti-inflammatory activities and stimulates angiogenesis and wound healing [28, 29].

Studies employing phototherapy to improve wound healing in CVU patients have demonstrated contradictory or unclear results. Landau et al. (2010) demonstrated that phototherapy with a broadband ranging from 400 to 800 nm could improve the healing rate (decreased time to close ulcers) in treated patients in relation to the placebo group [12]. Another study applied phototherapy in the red (660 nm) and infrared (890 nm) spectrum simultaneously, twice a week, over 90 days [13]. The authors also found an increased healing rate in irradiated ulcers, in relation to the placebo and control groups, as early as 30 days into the treatment [13]. However, the main limitation of this study was the low number of non-treated (placebo and control) ulcers ($n=6$ and 7, respectively). On the other hand, other authors did not find any beneficial effects of infrared (940 nm) light associated with a hydrocellular dressing applied weekly, in relation to the healing rate and decrement of ulcer areas over 9 weeks [30]. A clinical study, using laser diodes at 625 nm daily in the first week, followed by alternate days from week 2 up to 90 days, showed no effects of phototherapy on wound healing of CVUs in comparison to placebo treatment and control patients [31].

Lagan et al. (2012) irradiated CVUs with a laser probe with red (660 nm) and infrared (950 nm) diodes, over 4 weeks (once per week) and reevaluated patients after 8 and 12 weeks. The authors observed no effect of phototherapy on treatment and placebo groups during laser treatment. However, the areas of irradiated ulcers seemed to progressively decrease after ending the laser treatment, from weeks 8 to 12 [32], suggesting phototherapy may present a delayed effect on stimulating tissue repair. Our results also suggest that LED therapy may have late beneficial effects on the healing process, since the areas of irradiated ulcers did not increase after the 19th week and presented a significant difference in relation to the relative areas of the control group. Although we did not find early effects such as those reported by Caetano et al. (2009), we investigated whether phototherapy could inhibit the local inflammatory response, preventing later expansion of ulcer areas.

In order to investigate the effects of LED therapy on local inflammatory reaction, we evaluated the levels of TNF-α in ulcers exudates. TNF-α is an inflammatory cytokine, and it has been associated with poor wound healing [9]. The TNF-α activates inflammatory cells, stimulates degradation of extracellular matrix, induces oxidative stress, and impairs fibroblast proliferation and matrix synthesis [9, 33]. It can be isolated from active CVUs, and its levels decrease during the healing phase [11]. Inhibition of TNF-α using neutralizing antibodies improved wound healing in experimentally induced CVUs and in CVUs resistant to conventional treatments [10, 9]. In a study using animals, blocking TNF-α reduced local inflammatory cell infiltration and inflammatory cell signaling, thereby improving tissue repair [9]. Phototherapy has been proven

to down-modulate TNF-α production and decrease local infiltration of inflammatory cells in soft tissues [17, 34, 35]. However, no significant decrease in TNF-α was observed during the healing process in our study nor were differences detected between the control and LED ulcers, suggesting LED therapy has no evident effect on modulation of local TNF-α levels in CVUs.

Other mechanisms may be associated with the late effects of phototherapy on CVUs, such as an improvement in fibroblastic proliferative responses [19], inducing angiogenesis [36, 37], inhibition of matrix metalloproteinases (MMP-2, MMP-3, MMP-9, and MMP-13) [35, 37], and stimulating growth factor expression [36, 37]. Recently, it has been demonstrated that non-healing, resistant to treatment CVUs, overexpresses MMPs during later phases (after 60 days of appropriate treatment) of wound healing [22]. Since phototherapy decreases MMP activity [37], it may improve the later healing process in refractory ulcers. Indeed, further studies must address which and how other biological effects of phototherapy may be associated with the decreased number of non-healing ulcers in patients receiving LED therapy.

Conclusion

We conclude that LED therapy at 625 nm may have delayed effects on improving wound healing and decreasing ulcer resistance to Unna's boot treatment. However, LED therapy did not accelerate wound repair and did not inhibit local production of TNF-α.

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