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Effect of topical haemoglobin on venous leg ulcer healing

ABSTRACT

Background: The improvement of oxygenation is gaining increasing attention as an important aspect in modern wound care. The aim of such complementary care is to improve and accelerate wound healing.

Patients and Methods: A solution comprised of purified haemoglobin was added to the standard wound care of subjects with a venous leg ulcer and compared to a control group administered a saline solution without haemoglobin. Each group consisted of 36 randomly chosen patients. The duration of the treatment was 13 weeks and the primary end point was the reduction of wound size or wound closing.

Results: In the group treated with the haemoglobin solution, an average wound size reduction of 53% was obtained, whereas no statistically significant reduction in wound size was observed in the control group.

Conclusion: The addition of a haemoglobin solution in the wound care of venous leg ulcers resulted in a marked improvement of wound healing compared to the control group.

Keywords: haemoglobin, oxygen, venous leg ulcer, wound healing

INTRODUCTION

Chronic wounds are wounds that show no tendency to heal after 8-12 weeks[1,2]. The incidence of chronic wounds has recently increased, particularly in older patients, with venous leg ulcers being one of the most common chronic wounds[1]. In many cases, prolonged oxygen deficiency (hypoxia) to the skin and subcutaneous tissues is associated with chronic wounds[3,4]. Tissue hypoxia is considered a common aetiology for the pathological processes in wound healing disorders, particularly in patients with peripheral arterial occlusive disease (pAOD), chronic venous insufficiency (CVI), and diabetes mellitus[4,5,6,7,8,9].

Oxygen is vital for wound healing, as more oxygen is needed for a large number of processes during all phases of wound healing (inflammation, granulation, neoangiogenesis, re-epithelisation, and tissue reorganisation)[10,11,12]. Therefore, improving the oxygen supply to chronic hypoxic wounds in particular becomes increasingly important [3,10,11,12,13,14]. In addition to treating underlying diseases, such as pAOD, CVI, or diabetes mellitus, further medical treatment approaches such as local normobaric and systemic hyperbaric oxygen treatment as well as other topical treatments with subsequent oxygen release are used in clinical settings[8,14,15,16].

One principle means of supplying necessary oxygen to a chronic wound is external administration. However, this is precluded by the exudate from the wound bed, which is a very effective diffusion barrier to oxygen. As moist treatment of chronic wounds is the current treatment standard[5,6,7], concepts for improving oxygen diffusion have been investigated. To this end, Barnikol et al.[17] exploits the principle of haemoglobin-mediated facilitated oxygen diffusion in aqueous solutions by applying haemoglobin to the wound bed as an aqueous solution. In addition to the free diffusion of oxygen, which is otherwise limited by the fluid barrier, the addition of haemoglobin possibly results in considerably improved facilitated diffusion[18]. Carrier molecules that are well-suited for this include mammalian haemoglobins, which are water-soluble and are capable of transporting oxygen outside of red blood cells[17,18,19] (Fig. 1). ▶

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Conflict of interest: Some material and figures has been provided by SastoMed

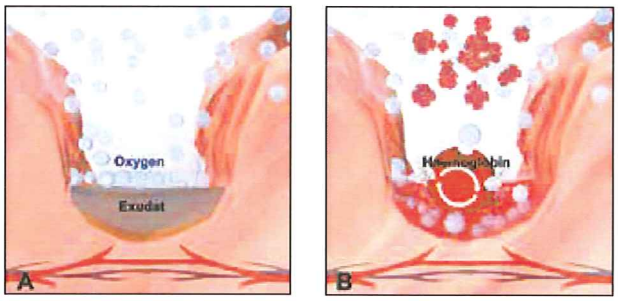


Fig. 1: Haemoglobin as an oxygen transporter
(A) The wound exudate presents a barrier to oxygen exchange, effectively blocking any external oxygen supply to the wound.
(B) The haemoglobin applied via the spray is evenly distributed throughout the wound exudate, binds ambient oxygen, and transports it to the wound bed (based on the principle of facilitated diffusion)[18].

This study investigated the effect of a haemoglobin solution on wound healing of venous leg ulcers for a period of 13 weeks compared with a control group that did not receive the haemoglobin solution. The results confirm the therapeutic potential of the haemoglobin solution to improve wound healing when treating patients with venous leg ulcers.

MATERIAL AND METHODS

Haemoglobin solution

The purified haemoglobin used in this study was produced from porcine blood and formulated as an aqueous 10% solution (10% carbonylated haemoglobin, 0.7% phenoxyethanol, 0.9% NaCl, 0.05% N-acetylcysteine, 88.35% water). After virus-removal filtration and sterile filtration, the solution was provided as a ready-to-use spray in bag-on-valve canisters. This haemoglobin spray supplies the wound bed tissue with oxygen via diffusion for an extended period of time[17,20] and does not contain any pharmaceutically active ingredients; its mode of action is based only on physical oxygen transport. The haemoglobin spray was applied to the wound bed following meticulous wound cleaning and disinfection. Subsequently, the wound was dressed with a thin, air-permeable nanofibre textile.

Study design

This clinical study was a prospective, randomised, single-blind, monocentric study embedded in grant study no. IGA NS/10093-4/2008 sponsored by the Czech Ministry of Health. The Ethics Committee of Prague 10 Faculty Hospital gave a positive opinion for the grant study.

The overall sample size calculation was based on comparison of wound size between the haemoglobin therapy arm and the placebo and was performed using East 5.4 software under the assumption that a group sequential design would be implemented with one final analysis for the wound size. The following assumptions were made when estimating the required sample size:

- A 1:1 randomization scheme for haemoglobin therapy and placebo arms
- An overall 5%, two-sided risk of erroneously claiming the superiority of haemoglobin therapy in the presence of no true underlying difference (i.e. overall Type I error)
- A 90% chance of successfully claiming superiority of haemoglobin therapy in the presence of a true underlying difference (i.e. Type II error).

In order to show a 20% reduction in wound size in the haemoglobin arm versus the placebo in the final analysis, the required number of patients to achieve 90% power for rejecting the null hypothesis if the alternative hypothesis is true is 72. To achieve this, an estimated total of 72 subjects (36 subjects in each arm) would need to be enrolled, leading to implementation of final analyses 13 weeks after the start of the study. The study presented here consists, therefore, of two groups with a total of 72 subjects recruited over a period of 4 months.

Thirty-six patients were included in Group 1 (haemoglobin group). Application of the haemoglobin solution was integrated into the treatment regimen before the wound was covered with a dressing. The 36 subjects in the comparator group (Group 2) were treated analogously to the Group 1 subjects, but without application of the haemoglobin solution.

Based on clinical experience, the primary objective of this study was to investigate the effect of the haemoglobin spray on the size of the wound surface area during the 13-week treatment period. The safety of the treatment was evaluated as the secondary parameter.

The attending doctor whom evaluated the wound surface area and assessed the condition of the wound were blinded. In contrast, the nurses involved in treatment and wound care were not blinded.

Inclusion criteria: Patients were >18 years of age, had a venous leg ulcer a minimum of 1.6 cm in all directions and a maximum wound surface area of 50 cm^2 persisting for >8 weeks. Patients had an ankle brachial index (ABI) of more than 0.8 to rule out an arterial cause of the ulcer. Further diagnostic procedures for precise characterisation of the patients were colour duplex ultrasonography and measurement of pedal pulses with normal arterial values.

Exclusion criteria: Vasculitis, non-venous leg ulcer, treatment with systemic antibiotics, corticosteroids, or other oral immunosuppressants before or during course of the study, and pregnancy.

Treatment

Each subject received compression therapy during the day according to the current guidelines in the Czech Republic based on previous clinical experience; compression therapy was initiated 2 weeks prior to study inclusion. Although there were no particular specifications for the compression bandage, the same compression bandages (Ideal/Hartmann) were used for all patients, which were applied at a constant circumference at the ankle to ensure adequate compression pressure. Continuation of the compression therapy over the entire treatment period was an integral element of the treatment and was monitored correspondingly.

The subjects were hospitalised for the first 2 weeks of treatment, which corresponds to the average hospitalisation time of patients with venous leg ulcers. After this period, the subjects were treated at home by study nurses.

The wound dressings were changed daily according to the standardized study protocol. After cleaning with saline, the haemoglobin solution was sprayed onto the wounds of the Group 1 subjects. In Group 2, the wound was treated in the same way as in Group 1, but a 0.9% saline solution (without haemoglobin) was applied in place of the haemoglobin solution. The wounds were then dressed with a nanofibre dressing used at the hospital (Nanotextile, Elmarco, Liberec, Czech Republic) and fixed with gauze. Subjects wore their compression bandage during the day, but not at night, according to the guidelines of the Czech Republic, in order to avoid an additional burden to patients.

After the study ended, patients were asked to visit the investigator once a week for a follow-up examination. If the wound had not fully closed by the end of the study, patients were offered continuing treatment; several patients (11 in Group 1 and 31 in Group 2) took advantage of this offer.

Analysis of wound surface area changes

The wound margins were marked on a triple-layer transparent sterile film, electronically scanned, and the wound surface area measured by computer-aided analysis. The surface area on the day of inclusion in the study (T=0) was used as a baseline. Thereafter, the surface area was measured after wound cleansing on days 7, 14, 21, 28, 35, 42, 49, 56, 63, 70, 77, 84, and 91. Wound sizes in both treatment groups were analysed statistically by analysis of variance (ANOVA) to determine whether the differences before and after treatment were significant. Post-hoc Bonferroni tests were applied to highly significant differences (i.e. $p<0.001$).

Furthermore, the quality of the wound was assessed during the regular check-ups, and parameters such as wound coating (necrotic tissue, fibrin coating), granulation, epithelisation, and pain relief using a visual analog scale (VAS) (0=pain-free, 10=maximum imaginable pain) were recorded[21]. Photographs were taken of the wounds at inclusion and over the course of treatment.

Table 1: Summary of demographic data of the study subjects

	Group 1 (haemoglobin)	Group 2 (control)
No. subjects	N=36	N=36
Drop outs	2	5
Average age (years)	65	59
Female (%)	69.4	58.3
Average duration of ulcer (range)	2 years (3 months-6 years)	2 years (3 months-6 years)
Average ulcer size at start of study (cm^2)	18.7±9.9	17.5±9.3

Adverse events

The subjects' safety was ensured during the study according to the European standards for clinical trials on medicinal devices in human subjects. The causality of adverse events (AE) was defined as an event a) caused by the treatment, b) possibly caused by the treatment, or c) independent of the wound treatment given with/without haemoglobin. All AEs that occurred during the study were immediately reported and documented.

RESULTS

Group demographics

Seventy-two patients (64% female and 36% male) were included in this study and divided into 2 groups of 36 subjects each (Table 1). The mean age of the subjects in Group 1 (69% female, 31% male) was 65 years and in Group 2 (58% female, 42% male) was 59 years. The mean period over which the treated chronic wounds had persisted was 2 years (3 months–6 years). Of the 72 patients included in the trial, 65 were treated for the entire period of 13 weeks. In Group 1, two of the subjects could not be treated for the whole study period: one was admitted to the hospital due to liver disease, and another had to discontinue treatment due to a severe wound infection. In Group 2, one subject was excluded after 9 weeks due to non-compliance and another four asked to discontinue the treatment prematurely after 9 or 10 weeks because they did not respond to the treatment.

Changes in wound surface area

Changes to the wound surfaces were examined over the treatment period of 13 weeks in both groups (Figs. 2A-C). The mean wound surface area of the subjects in Group 1 was 18.7 cm^2 at study inclusion. During the 13 weeks of treatment, the mean reduction in the wound surface area was 53.4% ($p<0.0001$). Overall, 33 subjects showed

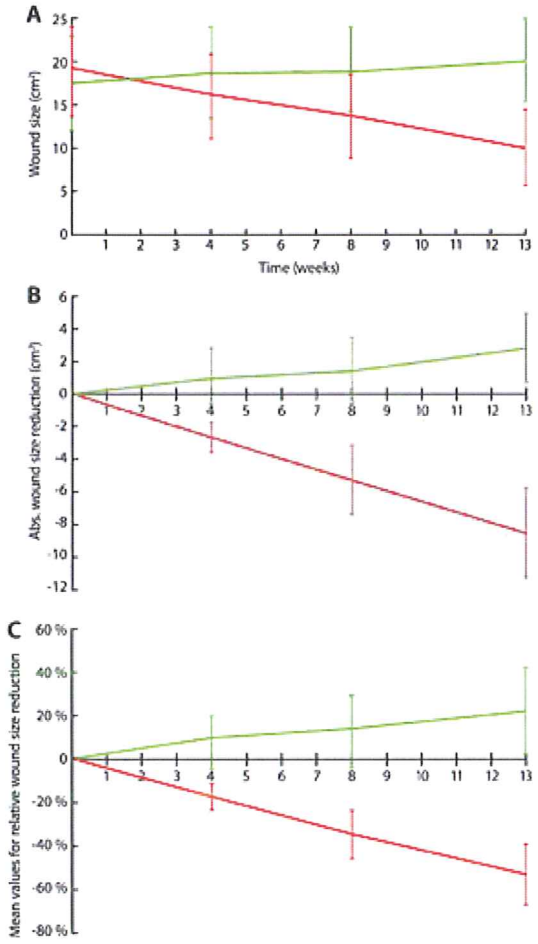


Fig. 2: Mean change in wound size over 13 weeks.
Group 1 (—) is the treatment group (haemoglobin solution applied) and Group 2 (—) is the control group (0.9% NaCl solution applied). The wound surface area (cm²) in both groups was observed for 13 weeks and measured at the start of the study and after 4, 8, and 13 weeks of treatment.
The analysis of the change in the wound surface area showed a continuous and statistically significant reduction in the wound surface area for Group 1 (week 0=18.6 cm² and week 13=10.2 cm², p<0.0001), while Group 2 showed a slight increase in wound surface area (week 0=17.5 cm² and week 13=20.2 cm²). Significant differences between the two groups are also shown (p values are indicated).
(A) Change in the mean wound surface area (cm²).
(B) Reduction in the wound surface area (cm²).
(C) Mean values of relative wound reduction (%). The mean wound reduction in Group 1 was 53% and the mean wound increase in Group 2 was 21%.
NS=not significant

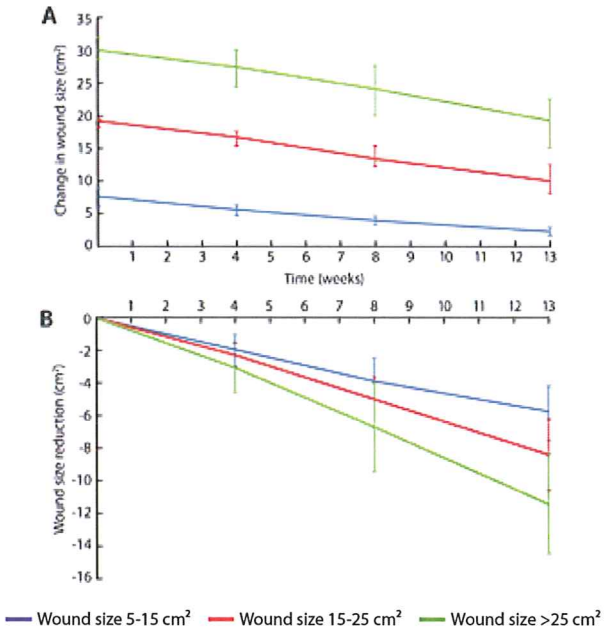
a positive tendency to heal, with a significant reduction in the wound surface area over the study period. One of these 33 subjects achieved complete wound closure after 12 weeks of treatment, while one subject exhibited a slight enlargement of the wound surface area over the entire observation period (10.4%). The mean wound surface area of the subjects in Group 2 was 17.5 cm² at inclusion. In this group, a mean enlargement of the wound surface area to 20.2 cm² was observed over the treatment period.

Overall, 14 of the 31 subjects treated over the entire study period showed a slight reduction in wound surface area (mean and SD of the difference was 88% and 13%, respectively); in contrast, 17 subjects showed an increase in wound surface area (mean and SD of the difference was 139% and 38%, respectively). Comparison of the average mean wound size reduction between both groups at the beginning and end of the study revealed a statistically significant reduction in wound size in Group 1 at the end of the study (p>0.0001).

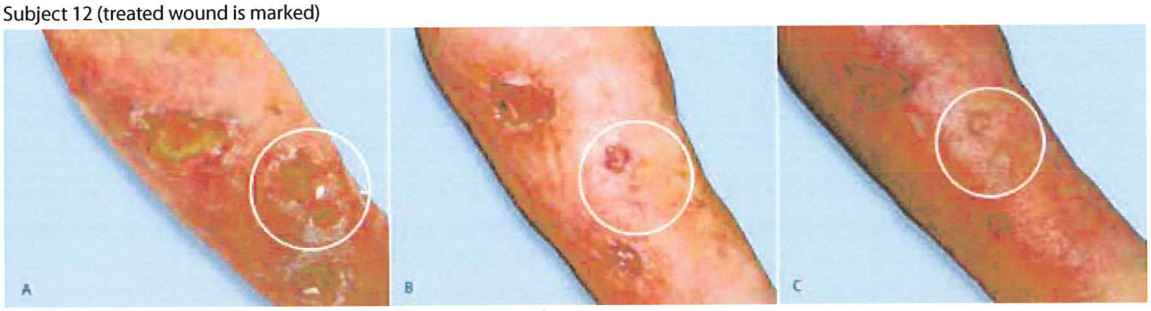
Effect of wound surface area on healing speed

The mean reduction of the wound surface area in Group 1 over the entire treatment period was 53% (Fig. 2C). In order to obtain a more detailed analysis of the effect of the change in wound surface area on healing speed over the treatment period, the data from Group 1 were divided into three subgroups according to initial wound size (5–15 cm², >15–25 cm², and >25 cm²), and the decrease in surface area over time was determined (Fig. 3A and B). All three subgroups showed a parallel and constant decrease over the entire 13-week period (Fig. 3A). Figure 3B shows the absolute values of the reduction in surface area of the three subgroups in cm². The mean value of absolute reduction in surface area after 13 weeks was 11.5 cm² for wounds that had been larger than 25 cm², and 8.5 cm²

Fig. 3: Changes in wound surface area of three subgroups of Group 1 based on the wound surface area at study start.
Group 1 was divided into the following sub-groups:
a) 5-15 cm² (—), b) >15-25 cm² (—), and c) >25 cm² (—).
The graphs show the changes in wound surface areas and the absolute figures for the reduction in the surface area (mean ±SD).
(A) Change in wound size over 13 weeks (cm²) showing a continuous reduction in size over time.
(B) Mean decrease in wound size over 13 weeks (cm²) showing a larger reduction in surface area for large wounds from baseline than for small wounds.



Subject 12 (treated wound is marked)



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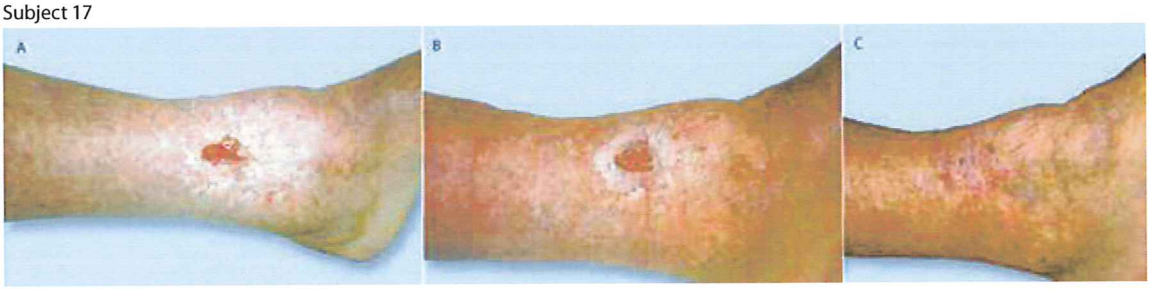

Fig. 4: The course of wound healing over 13 weeks of two subjects in Group 1. (A) Wounds at the start of the study. (B) Wounds after 6 weeks. (C) Wounds upon completion of the study.

for wounds initially between 15 and 25 cm². Wounds that were smaller than 15 cm² showed a mean reduction of 5.7 cm². The resulting mean reduction in wound surface area over the 91-day period was 6.3 mm²/day for wounds measuring 5-15 cm², 9.3 mm²/day for wounds measuring >15-25 cm², and 12.6 mm²/day for wounds >25 cm². Taking all the wounds treated into account, this corresponds to a 9.3 mm² reduction in wound surface area per day. As an example, Figure 4 shows the course of wound healing over 13 weeks for two subjects in Group 1.

Wound quality

Additional clinical aspects of wound healing in venous leg ulcers in the two groups were evaluated to determine the quality of wound healing. The parameters described above in "Treatment" (necrotic tissue, fibrin coating, granulation, and epithelisation) were recorded on days 0, 15, 42, and 91. By day 91, the subjects in Group 1 had a marked reduction in necrotic tissue (48%) and fibrin coating (42%), and a marked increase in granulation tissue (75%) and epithelisation (78%) compared to mean values on day 0. In contrast, the subjects in Group 2 had a 17% and 12% reduction in necrotic tissue and fibrin coating, respectively, whereas granulation tissue increased by 18% and epithelisation by 7% from day 0 to day 91.

The subjects in both groups also rated pain intensity on a VAS on days 0, 15, 42, and 91. The subjects in Group 1 showed a mean reduction in pain intensity of 68% ($p<0.01$) from day 0 (VAS=5.8) to day 91 (VAS=0.1). The reduction in pain intensity for subjects in Group 2 were 7% ($p>0.05$) from a mean baseline value of VAS=5.1 on day 0 to VAS=4.8 on day 91 (Fig. 5).

Adverse events

One subject in Group 1 was admitted to hospital with liver disease, which was unrelated to the treatment. Four cases of AEs were reported in Group 2. One subject complained of a burning sensation in the wound, one had rhinitis, and two developed a mild headache. However, these events were classified as being independent of the treatment and did not lead to drop-out from the study.

DISCUSSION

Chronic wound healing is a complex process in which different phases of wound healing interact[10,11,12]. One essential factor in the wound healing process is an adequate oxygen supply. However, many chronic wounds have inadequate oxygen perfusion due to underlying diseases such as CVI or pAOD, among others, leading to tissue hypoxia. If such a hypoxic state persists for a prolonged period of time, a wound healing disorder usually occurs if a wound develops. In these cases, many normal processes are either retarded or stagnated, including pathogen defence, cell proliferation during the granulation or epithelisation phases, or synthesis of the extracellular matrix[3,10,11,12].

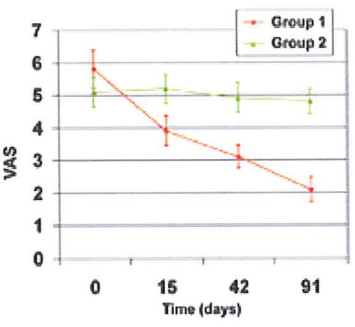

Fig. 5: Assessment of pain intensity. Subjects in both groups rated pain intensity on days 0, 15, 42, and 91 using a VAS. A marked decrease in VAS among subjects treated with the haemoglobin spray (Group 1) can be observed in comparison to the control group (Group 2).

In addition to mandatory treatment of the primary disease to revascularise the tissues, an external oxygen supply to the wound is of great interest in the field of wound treatment[3,8,13,14,15,16,17]. Here, we used a haemoglobin solution to treat wounds based on the premise that haemoglobin improves oxygen transport through the wound exudate to the wound bed by facilitated diffusion[20, 21]. In this study, patients with a venous leg ulcer that, despite prior *lege artis* wound care, did not show any significant improvement, were included, whereas patients with chronic wounds of a different aetiology were not included in the interest of group homogeneity. However, it may be expected that patients with arterial occlusive disease, for instance, would also respond positively to haemoglobin therapy, particularly patients with critical limb ischemia[22,23], but further studies are needed.

Though compression therapy was used during the study for all subjects, the effect of the therapy on the observed haemoglobin treatment results was minimised by the fact that both groups had already been treated with compression therapy before the start of the study, and continued the treatment consistently during the study.

We found that the majority of the subjects in Group 1 treated with the haemoglobin solution had a significant and continual healing tendency. The continual healing tendency was independent of the initial wound surface area size, and was comparable in the three wound size cohorts analysed. In addition to the marked reduction in wound surface area, the subjects in Group 1 also showed a reduction in scab volume and fibrin coating, and a marked increase in granulation tissue and epithelisation. Wound pain also decreased over the course of the study in Group 1 subjects.

We demonstrated that a haemoglobin solution can be used easily and safely to treat venous leg ulcers, results that are supported by case reports[17] and an open market authorisation study in Mexico[22]. Haemoglobin can stimulate wound healing and have a potent adjunctive effect on the healing process. The treatment described here is a suitable alternative or adjunct to other topical forms of wound treatment as it is effective and causes no undesirable side effects. Furthermore, topical use of haemoglobin for a broader spectrum of medical indications is conceivable. Clinical studies including different wound types are needed to further support the emerging evidence of the broad benefit of haemoglobin adjunct therapy in wound care.

SUMMARY:

The amount of oxygen supplied to a wound plays an important role in successful treatment of chronic ulcers of the lower limb, such as venous leg ulcers. Therefore, the oxygen supply to the wound bed is an important add-on procedure for successful wound treatment. This study demonstrated the positive effect of topical administration of a haemoglobin solution on wound healing in patients with a venous leg ulcer.

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