Effect of topical haemoglobin on healing in patients with venous leg ulcers

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Abstract

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

**Patients and Methods:** A solution comprising purified haemoglobin was added to the standard wound care procedure for subjects with venous ulcer and compared to a second group without the addition of the haemoglobin. In each group, 36 patients were included. The duration of treatment was 13 weeks. Primary end point was reduction of wound size or wound closing.

**Results:** In the group treated with the additional haemoglobin solution, an average of 53% wound size reduction was obtained. No statistically significant reduction was observed in the second group.

**Conclusion:** The addition of haemoglobin solution in the wound care procedure for leg ulcers showed a marked improvement of wound healing in comparison to a control group.

**Running head:** Haemoglobin promotes healing of venous ulcer

**Keywords:** Haemoglobin, oxygen, wound healing, venous leg ulcer
Introduction

Chronic wounds are defined as wounds that show no tendency to heal after 8-12 weeks [1, 2]. With increasing age in particular, an increasing incidence of chronic wounds has been observed. Venous leg ulcers are one of the most commonly observed chronic wounds [1]. A prolonged deficient oxygen supply (hypoxia) to the skin and the subcutaneous tissues is associated with chronic wounds in many cases [3, 4]. Tissue hypoxia is considered to be 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]

Since 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]), oxygen assumes central importance for wound healing. Therefore, improvement of the oxygen supply to chronic hypoxic wounds in particular will become of increasing importance [3, 10, 11, 12, 13, 14].

Besides causal treatment of the 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, 15, 16].

One principle means of supplying the oxygen needed by a chronic wound is its external administration. However, this is precluded by the exudate from the wound bed which is a very effective diffusion barrier to oxygen. Since moist wound treatment of chronic wounds is the current treatment standard [5, 6, 7], concepts for improving oxygen diffusion have been investigated. One approach towards achieving this was presented by Barnikol et al. [17]. This approach is based on the use of haemoglobin as an oxygen carrier, applied to the wound bed as an aqueous solution. This exploits the principle of haemoglobin-mediated facilitated oxygen diffusion in aqueous solutions.
Besides 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).

This study investigated the effect of a haemoglobin solution on wound healing for a period of 13 weeks compared with a comparator group without the use of 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 used
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 made up to 100% with water). After virus-removal filtration and sterile filtration steps the solution was provided as a ready-to-use spray in bag-on-valve canisters. The haemoglobin spray was sprayed onto the wound bed after meticulous wound cleaning and disinfection. Subsequently, the wound was dressed with a thin, air-permeable nanofibre textile.

The haemoglobin in the solution applied supplies the tissue in the wound bed with oxygen by diffusion for an extended period of time [17, 20]. The haemoglobin spray does not contain any pharmaceutically active ingredients while its mode of action is based only on physical oxygen transport.
Study design

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

The part of the grant study presented here consists of two treatment groups with a total number of 72 subjects.

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

The primary objective of the 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 subjects were treated for 13 weeks. The attending doctor evaluated the wound surface area and assessed the condition of the wound. The doctor maintained the blinding to make independent assessment of the wound surfaces possible. By contrast, the nurses involved in the treatment and wound care remained unblinded.

The patients included in the study were recruited over a period of 4 months.

Inclusion criteria: Patients aged 18 and above who had had a venous leg ulcer for more than 8 weeks were included in the study.

For inclusion in the study the ulcers had to have a minimum dimension of 1.6 cm in all directions and a maximum wound surface area of 50 cm². Another parameter was 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 measurements of the pedal pulses with normal arterial values.
Exclusion criteria were vasculitis, non-venous leg ulcer, and treatment with systemic antibiotics, corticosteroids or other oral immunosuppressants before or in the course of the study. No pregnant patients were included in the study.

Treatment

Each subject received compression therapy during the day. Compression therapy was initiated 2 weeks prior to study inclusion. Although there were no particular specifications for the compression bandage, continuation of the compression therapy over the entire treatment period once started was an integral element of the treatment and was monitored correspondingly. The compression bandages chosen had to make it possible to apply adequately high compression pressure. This was ensured by a constant circumference at the ankle.

The subjects were hospitalised for the first 2 weeks of treatment and then treated at home by study nurses. The wound dressings were changed daily. After cleaning the wound the haemoglobin solution was sprayed onto the wounds of the Group 1 subjects. In Group 2 the wound was treated with a 0.9% saline solution (liquid without haemoglobin). The wounds were then dressed with a dressing used at the hospital (Nanotextilie, Elmarco, Liberec, Czech Republic). During the day the subjects wore their compression bandage. At night the compression bandage was removed and the nanofibre wound dressing fixed.

After completion of the study the patients were asked to visit the investigator once a week for a follow-up examination. Furthermore, the patients were offered continuing treatment after completion of the study if the wound had not closed fully. Several patients (11 in Group 1 and 31 in Group 2) took advantage of this offer.
Analysis of the wound surface area changes

The wound margins were marked on a triple-layer transparent sterile film and electronically scanned, and the wound surface area was measured by computer-aided analysis. The surface area on the day of inclusion in the study (T=0) was taken as baseline and measured again after cleaning the wound on Days 7, 14, 21, 28, 35, 42, 49, 56, 63, 70, 77, 84 and 91. The sizes of the wounds 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 tissue, epithelisation and pain relief using a VAS (0 was pain-free, 10 was maximum imaginable pain) were recorded [21]. Photographs were taken of the wounds at inclusion and during the course of treatment.

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: a) an event caused by the treatment -, b) possibly caused by the treatment - or c) independently of the wound treatment given with/without haemoglobin. All AEs that occurred during the study were immediately reported and documented.

Results

Demographics of the two groups

Seventy-two patients (64% female and 36 % male) were included in this study and divided into 2 groups of 36 subjects each. The mean age of the subjects in Group 1 (69% female,
31% male) was 65 years and 59 years in Group 2 (58% female, 42% male). The mean period over which the chronic wounds we treated had persisted was 2 years (3 months to 6 years). 65 of the 72 patients included in the trial were treated for the entire period of 13 weeks.

From the comparator group, one subject was excluded after nine weeks due to non-compliance. Another four subjects from this group asked to discontinue the treatment prematurely after 9 and 10 weeks respectively because they did not respond to the treatment.

In the haemoglobin group, two of the subjects could not be treated for the whole study period. One subject was admitted to hospital due to liver disease, and another had to discontinue treatment because of a severe wound infection.

Changes in the wound surface areas

The 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 (haemoglobin group) was 18.7 cm² 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 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' treatment. Only 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 (comparator group without haemoglobin) 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 who were treated over the entire study period showed a slight reduction in the wound surface area; by contrast 17 subjects showed an increase.
Effect of the wound surface area on the speed of healing

The mean reduction of the surface area of the wounds in Group 1 (with haemoglobin) over the entire treatment period was 53% (Fig. 2C).

For a more detailed analysis of the effect on the change in the wound surface area over the treatment period, the data from Group 1 were divided into three subgroups with the wound size ranges 5 - 15 cm², >15 – 25 cm², and >25 cm².

Figures 3A and 3B are graphs of the changes in the wound surface areas, i.e. the decrease of the areas over the entire treatment period.

All three subgroups show 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 25cm², and 8.5 cm² for wounds initially between 15 and 25cm². Wounds that were smaller than 15cm² 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 every day. Figure 4 shows the course of wound healing over 13 weeks for two subjects in Group 1 as an example.

Wound quality

Additional clinical aspects of wound healing in venous leg ulcers in the two test groups were evaluated to determine the quality of wound healing. The parameters described above were recorded on Days 0, 15, 42 and 91.
By Day 91 the subjects in Group 1 who were treated with haemoglobin showed a marked reduction in necrotic tissue (48%) and fibrin coating (42%), while a marked increase in granulation tissue (75%) and epithelisation (78%) was observed compared to the mean values on Day 0.

By contrast, the subjects in Group 2 (without haemoglobin) showed a 17% reduction in necrotic tissue and a 12% decrease in fibrin coating, 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 = 2.1). The reduction in pain intensity for the subjects in Group 2 amounted to 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**

No adverse events were observed in connection with the study. One subject in Group 1 was admitted to hospital with liver disease. However, the disease was not related to the treatment.

Four cases of adverse events were reported in Group 2. One subject complained of a burning sensation in the wound, one had rhinitis, and two subjects developed mild headache. However, these events were classified as being independent of the treatment and did not lead to drop-out.

**Discussion**

Chronic wound healing is a complex process in which different interleaving phases of wound healing interact [10, 11, 12]. One essential factor for the wound healing process is an adequate oxygen supply during all the phases of wound healing. However, in many chronic wounds we find inadequate perfusion and thus hypoxia of the tissues due to the underlying
diseases such as chronic venous insufficiency or peripheral arterial occlusive disease. If such a hypoxic state persists for a longer period of time this usually results in a wound healing disorder if a wound develops. Processes such as defence against pathogens, cell proliferation during the granulation or epithelisation phases, or the synthesis of the extracellular matrix, are either retarded or stagnate completely [3, 10, 11, 12].

Besides mandatory treatment of the primary disease to revascularise the tissues, an additional supply of oxygen to the wound is also of great interest in the field of wound treatment [3, 8, 13, 15, 16, 17].

Patients with a venous leg ulcer were included in the study presented here. Despite prior leges artis wound care, the wounds did not show any significant improvement. One effect of compression therapy on the observed treatment results was minimised by the fact that both groups had already been treated with compression therapy before study start, and that this treatment was also continued consistently during the study.

Patients with chronic wounds with a different aetiology were not included in this study in the interest of group homogeneity. However, it may be expected that patients with arterial occlusive disease, for instance, could also respond positively to haemoglobin therapy. Although wound treatment was comparable in both groups, in one group a haemoglobin solution was applied to the wound after cleaning it and before dressing it. The haemoglobin serves to improve oxygen transport through the wound exudate to the wound bed by facilitated diffusion [18, 19].

The data show that the majority of the subjects in Group 1 who were treated with the haemoglobin solution revealed a significant and continual healing tendency. It was shown that the continual healing tendency was independent of the size of the wound surface area when starting treatment, and that it was comparable in the three wound size cohorts analysed.

The study presented here also recorded additional clinical aspects of wound healing in venous leg ulcers in the two test groups. Besides the marked reduction in wound surface area, the subjects in Group 1, who were treated with haemoglobin, also showed a reduction
in scab volume and fibrin coating, and a marked increase in granulation tissue and epithelisation. Wound pain also decreased. Furthermore, no adverse events (AE) were observed.

In addition to other case reports [17] and an open marketing authorisation study in Mexico [20], this study also demonstrated the safe and simple use of a haemoglobin solution in the treatment of venous leg ulcers. The results illustrate stimulation of wound healing and 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. It is effective and causes no undesirable side effects. Furthermore, the topical use of haemoglobin for a broader spectrum of medical indications is conceivable.

Summary:

The amount of oxygen supplied plays an important role for 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. The study proved the positive effect of the topical administration of a haemoglobin solution on wound healing in patients with a venous leg ulcer.

Acknowledgement

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Literature

Figures

Fig. 1: Haemoglobin as an oxygen transporter

The wound exudate presents a barrier to oxygen exchange so there is barely any external oxygen supply to the wound (1A). The haemoglobin is evenly distributed throughout the wound exudate, binds the ambient oxygen, and transports it to the wound bed – principle of facilitated diffusion [18] (1B).
Fig. 2: Mean change in wound size over 13 weeks

Group 1 = treatment group with additional haemoglobin solution, Group 2 = treatment group without additional haemoglobin solution. The wound surface area is given in cm². The wound surface areas in both treatment groups with and without added haemoglobin were observed for 13 weeks. The wound surface area was measured at study start 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 – week 13 = 10.2 cm², p< 0.0001), while Group 2 showed a slightly increasing tendency (week 0 = 17.5 cm² – week 13 = 20.2 cm²).

2A) Change in the mean wound surface area (cm²)
2B) Reduction in the wound surface area in cm²
2C) Mean values of relative wound reduction (%)

The mean wound reduction in Group 1 was 53%. By contrast, there was a mean increase in wound size of 21% in comparator Group 2.
**A**

Wound size (in cm²)

Mean Group 1
(Haemoglobin group)
Mean Group 2
(Comparator group)

**B**

Abs. wound size reduction (in cm²)

Mean Group 1
(Haemoglobin group)
Mean Group 2
(Comparator group)

**C**

Mean values for relative wound size reduction

Mean Group 1
(Haemoglobin group)
Mean Group 2
(Comparator group)
Fig. 3: Division of the group with the haemoglobin solution into three subgroups based on the wound surface area at study start: a) 5-15cm², b) >15-25cm² and c) >25cm². The graph shows the changes in wound surface areas and the absolute figures for the reduction in the surface area. 3A shows a continuous reduction in the course. Fig. 3B shows a larger reduction in surface area for large wounds at baseline than for small wounds.

3A) Change in wound size over 13 weeks (cm²).

3B) Mean decrease in wound size over 13 weeks (cm²).
Fig. 4:
Figure 4 shows the course of wound healing over 13 weeks for two subjects in Group 1 (with haemoglobin) as an example. The subjects’ wounds shown are at inclusion in the study (A), after 6 weeks (B), and on completion of the study (C).

a) Subject 12 (treated wound is marked)

b) Subject 17
Fig. 5: Assessment of pain intensity
Subjects in both groups rated the pain intensity on Days 0, 15, 42 and 91 on a VAS (Visual Analogue Scale). 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).