EFFECTS OF LOW-FREQUENCY ULTRASOUND ON MICROCIRCULATION IN VENOUS LEG ULCERS

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Abstract

Background: Therapeutic low-frequency ultrasound (US) has been used for many years to improve wound healing in chronic wounds like venous leg ulcers. No human data are available for the possible effects of single US applications on microcirculation and their frequency-dependency. Aims: To investigated the role of therapeutic low-frequency US on microcirculation of venous leg ulcers in vivo. Patients and Methods: This is a pilot study on an inpatient basis. We use a newly developed low-frequency continuous-wave US-equipment composed of a US transducer based on piezo-fiber composites that allow the change of frequency. In this study, we apply US of 34 kHz, 53.5 kHz, and 75 kHz respectively. Twelve patients with chronic venous leg ulcers are analyzed. As an adjunct to good ulcer care, therapeutic US is applied, non-contacting, once a day, in a subaqual position for 10 minutes. Microcirculation is assessed in the ulcers adjacent to skin before US-therapy, immediately after the treatment and 30 minutes later. We use a micro-light guide spectrophotometer (O2C, LEA Medizintechnik GmbH, Gießen, Germany) for calculation of blood flow velocity, hemoglobin oxygen saturation (SCO₂) and relative hemoglobin concentration (rHb) in 2 and 8 mm depth. Contact-free remission spectroscopy (SkinREM⁴, Color Control Chemnitz GmbH, Chemnitz, Germany) allows contact free measurements in the VIS-NIR range of the spectrum (400 ± 1600 nm). Results: It is seen that therapeutic US is well tolerated. One patient dropped out from a treatment series since he developed erysipelas responding to standard antibiotic. Effects were seen at 34 kHz only. The SO₂ values increased after single US application. The values for rHb were higher in the superficial layer of the wound bed (depth 2 mm) compared to deeper parts (8 mm depth). US treatment did not result in significant changes of rHb and blood cell velocity. The data obtained by remission spectroscopy disclose an increase of oxygenized hemoglobin. Conclusions: The major findings are that continuous-wave low-frequency US of 34 kHz, but not, 53.5 kHz or 75 kHz, has a temporary stimulatory effect on microcirculation mainly due to an improved oxygenation. Further studies with treatment series are necessary.

Key Words: Venous leg ulcers, microcirculation, low-frequency ultrasound, spectroscopy, hemoglobin, oxygen

Introduction

Therapeutic ultrasound (US) is used in medicine for different purposes. Based on frequency and power, one can differentiate various US types and applications:

- high-frequency therapeutic US (800-4000 kHz, 0.1-3.0 W/cm²; mesenchymal affections),
- medium-frequency US (500 kHz-10 MHz, bis 1500 W/cm²; surgery and oncology),
- low-frequency US (20-120 kHz, 0.05-1.0 W/cm²; wound treatment), and
- low-frequency power US (20-60 kHz, up to 200 W/cm²; dentistry and ophthalmic surgery.⁴⁻⁵

Chronic venous leg ulcers are a complication of chronic venous insufficiency, which is a great burden for patients, their family and the health care system. Leg ulcer treatment is dependent on vascular (phlebo-) surgery and wound management.⁶ A cornerstone of ulcer management is the wound bed preparation according to the TIME concept. TIME stands for debridement and removal of necrotic tissue and cell debris, control and treatment of infection and inflammation, moisture balance in the wound to avoid macerations of neighbouring skin and activation of keratinocytes from the wound edges.⁶ Since venous ulceration is accompanied by an inflammatory phase of disturbed microcirculation, another important effort would be the improvement of local microcirculation.⁶

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Therapeutic US has been used for debridement of chronic wounds.\(^7\) In the literature, one can find recommendations to use higher frequencies (about 3 MHz) with short distance effects for the more superficial wounds and lower frequencies (less than 1 MHz) for deeper wounds.\(^1,2\) The major effect on tissue of low-frequency US is a mechanical one but antimicrobial and cellular stimulatory effects have also been discussed.\(^1,2\)

Clinical investigations demonstrated an improved ulcer healing, in particular, a better granulation.\(^7\,11\) Better granulation needs better blood supply. Therefore, US could also be an option to target microcirculation. Using different biophysical methods, we investigated the role of therapeutic low-frequency US on microcirculation of venous leg ulcers in vivo.

**Patients and Methods**

**Patients**

The study has got an approval from the Ethical Committee of the Saxonian Physicians Chamber (Landesärztekammer), Dresden. Inpatients at the Department of Dermatology and Allergology have been enrolled for this trial. Inclusion criteria were presence of Chronic venous insufficiency (CVI) with chronic leg ulcer(s), and age above 18 years. Chronic leg ulcers were defined as ulcer that did not show signs of healing within six weeks despite adequate ulcer care. All patients had verbal and written information and gave a written consent.

Exclusion criteria were infection or systemic inflammation, thrombosis or thrombophlebitis, vascular surgery in the last 12 months, decompensated metabolic or heart disease, diabetes mellitus, vasculitis, arterial occlusive disease (Ankle-brachial pressure index < 0.5), bleeding disorders, anticoagulation, metal implants in the area of treatment, pregnancy and lactation, sensory neuropathy, and cancer (excluding basal cell carcinoma).

Therapeutic low-frequency US was given as an adjunct to good ulcer care.

**Methods**

Leg ulcers were photo documented. The ulcer area was measured using the VisiTrak\textsuperscript{®} device (Smith and Nephew Germany, Tuttlingen, Germany).\(^12\) The wounds were scored by the Wollina Wound Score (WWS).\(^13\) The following items are scored: Granulation, color and consistency.

Granulation and scoring points: no granulation at all 0, granulation 25% of the wound area 1; 50% of the wound area 2, 75% of the wound area 3, complete wound area 4.

Color and scoring points: pale 0, pink 1, bright-red 2. Consistency and scoring points: spongy 0, solid 1. The maximum total score is seven. The score has been evaluated before.\(^13\)

For US treatment, ulcer dressings were removed. We performed no cleaning of the legs before US application to avoid other mechanical influences than US itself. Patients were in a sitting position with legs immersed in a 1:5 mixture of water and an antiseptic with octenidine dihydrochloride (Octenisept\textsuperscript{®}, Schülke and Mayr, Norderstedt, Germany). Low-frequency US was applied, non-contacting, once a day in a subaqual position at 30-40 cm water depth [Figures 1a, b]. The bath temperature was 32-37° C. The bath was emptied and cleaned after every treatment.

**US-device**

We developed a new US-equipment (USWH-1) composed of a US transducer based on piezo-fibre composites (Smart Material, Dresden, Germany) and an US generator (IMM Engineer Bureau, Mittweida, German). This device generated continuous US in the frequency range of 20 kHz to 120 kHz, maximum power 100 W (i.e. 2W/cm\(^2\) on the probe) [Figure 1]. This allowed selecting frequencies in this experimental setting [Figure 1a]. We performed investigations with therapeutic US of 34 kHz, 53.5 kHz, and 75 kHz, respectively. The power we used herein was 60 W.

**Measurements in microcirculation**

We used two different technologies to assess microcirculation in the ulcers and in a distance of 10 cm from the ulcer edge. All measurements were performed.

![Figure 1: Energy distribution for (a) 53 kHz and (b) 75 kHz ultrasound](image-url)
before US-therapy after 10 minutes resting, immediately after the treatment and 30 minutes after treatment. For the arrangement of sensors [Figure 2]. We performed some measurements without US application to calculate the influence of changing the position of the leg in the sitting position from vertical to horizontal. The effect on microcirculatory parameters was <5%. A change from standing position to laying position, however, would have a more tremendous effect.

We used a micro-lightguide spectrophotometer (O2C, LEA Medizintechnik GmbH, Gießen, Germany). The device transmits continuous wave laser light (830nm; 30 mW) and white light (500-800 nm, 1 nm resolution; 20 W) to the tissue where it becomes scattered and collected on the skin surface by fibers of the probe. Visible white light consists of electromagnetic radiation of various wavelengths. Collected light is split into its spectral components using charge-coupled device array and converted into an electrical signal. The digitized signal is recorded on a personal computer and data are analyzed by comparison with pre-recorded deoxygenized and oxygenized hemoglobin spectra for calibration.

A laser-Doppler shift caused by moving erythrocytes is detected, analyzed and shown as blood flow velocity. The product of moving erythrocytes velocity ($v_i$) and the number of red blood cell with the same velocity ($N_i$) is used for the calculation of relative blood flow (flow) within the microcirculation ($\sum v_i x N_i = \text{flow}$).

White light is used for the detection of hemoglobin oxygen saturation ($\text{SCO}_2$) and relative hemoglobin concentration ($\text{rHb}$). $\text{SCO}_2$ is determined by the blood color. The $\text{rHb}$ value is measured by the amount of light absorbed by the tissue. Therefore, $\text{rHb}$ presents a hemoglobin concentration per tissue volume. Both, $\text{rHb}$ and velocity are expressed in arbitrary units (AU), $\text{SO}_2$ in percent. The device allowed a measurement in 2 and 8 mm depth depending on the sensor used. The technique has been used in various experimental settings and validations.[14,15]

Contact-free remission spectroscopy (SkinREM3, Color Control Chemnitz GmbH, Chemnitz, Germany): The experimental investigation was performed with a novel diodarray VIS-NIR spectrometer system SKINREM (ZEISS Jena and J and M Analytische Mess- und Analysentechnik Aalen, Germany) with a contact free measuring head. At the same time, measurements were taken in the VIS-NIR range of the spectrum (400 ± 1600 nm). During all measurements, any contact to the wound or skin surface was avoided. The radiation of a 20 W halogen lamp was transmitted by quartz fibres to the contact-free measuring head (diameter of the circular radiated area: 4 mm). The remitted light of the tissue was detected by two quartz fibers in the center of the illuminated area. By the special construction, the fiber optic measuring head detected the remitted radiation only. The distance of the measuring head to the tissue surface was adjusted to approximately 1 mm. The reproducibility of the arrangement of the order of 0.03% was tested by measuring the subsequent remittances of a Spectralon standard and calculating the mean difference within the whole spectral range. The standard deviation of the spectral method was below 2% in the spectral range from 600 to 1300 nm and was 4% in the range of the hemoglobin doublet peak.[13] The arrangement of probes allowed the measurement of adjacent areas by O2C and SkinREM3 [Figure 2].

Statistics

One-sided Mann-Whitney U-test was used. A $P$ value <0.05 was considered statistically significant.

Results

Clinical parameter and tolerance

Twelve patients with CVI and 14 chronic leg ulcers were recruited (8 males, one female). The age range was 46 to 87 years (mean 76.8 years). The leg ulcer pathology was solely varicose with or without a trauma the patient remembered (n=7) or postthrombotic (n=5). Microbiological swabs taken from the ulcers revealed a mixed flora with the three most common germs – *Pseudomonas aeruginosa*, *Enterococci* and *Proteus mirabilis*.

The leg ulcer area was between 2.7 and 414.6 cm$^2$ (mean: 68. cm$^2$). The WWS before treatment was 1 to 3 (mean 1.4). A single US application of course did neither change the WWS (gross morphology of wounds) nor the wound area.

The therapeutic US was well tolerated. None of the patients reported treatment-related pain or other adverse effects during US application. One patient dropped out from a treatment series since he developed erysipelas responding to standard antibiotic.

![Figure 2: Application of the measuring probes for microcirculatory analysis.](image-url)
Microcirculation

The $\text{SO}_2$ value of leg ulcers varied between 60 and 90%. In a pilot study, we saw the first measurable effects after an application time $> 5$ min to 10 min only. There was a tendency of higher $\text{SO}_2$ values after single US application at 34 kHz only [Table 1]. The $\text{SO}_2$ increase was seen immediately and lasted for at least 30 min after US treatment within the ulcers. The difference immediately after treatment was significant compared to pre-treatment values ($P = 0.031$). When we analyzed the subgroup of venous leg ulcers without dermatofibrosis only ($n = 5$), the difference reached higher significance ($P = 0.01$). Because of standard variations the difference of the 30 minute-values to pre-treatment values were non-significant ($P > 0.05$). In the neighboring skin there was either no measurable effect (34 kHz, 53.5 kHz) or even a slight decrease (with 75 kHz).

The values for rHb were higher in the superficial layer of the wound bed (depth 2 mm) compared to deeper parts (8 mm depth) within the ulcers ($P < 0.05$) but not and the surrounding skin. A single US treatment in the frequency range from 34 kHz to 75 kHz did not result in a significant change of rHb of venous leg ulcers. In the neighboring skin there was a temporary but non-significant increase of rHb at a frequency of 34 kHz ($P = 0.05$; Table 2). Higher intensity might show stronger effects but this has not been studied with this prototype US-device. Blood cell velocity was higher in the deeper layer compared to 2 mm depth. This differences reached a statistical difference in ulcers and peri-ulcer skin ($P < 0.05$). On the other hand, blood cell velocity remained unaffected by therapeutic US [Table 3].

The data obtained by remission spectroscopy disclose an increase of oxygenized hemoglobin, which was statistically significant for venous leg ulcers without dermatofibrosis ($P < 0.05$; Figure 3).

When we compare $\text{SO}_2$ values with $\text{HbO}_2$ double peaks the difference was highly significant ($P = 0.00003$).

Table 1: $\text{SO}_2$ measurements in venous leg ulcers and periulcer skin at 8 mm depth

<table>
<thead>
<tr>
<th>Frequency (kHz)</th>
<th>T1</th>
<th>T2</th>
<th>T3</th>
</tr>
</thead>
<tbody>
<tr>
<td>Ulcer</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>34</td>
<td>64.2 (5.2)</td>
<td>75.2 (4.9)</td>
<td>73.5 (10.0)</td>
</tr>
<tr>
<td>53.5</td>
<td>79.0 (14.5)</td>
<td>78.5 (13.1)</td>
<td>70.8 (10.5)</td>
</tr>
<tr>
<td>75</td>
<td>68.0 (11.3)</td>
<td>68.7 (10.0)</td>
<td>69.0 (10.6)</td>
</tr>
<tr>
<td>Periculcer skin</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>34</td>
<td>65.0 (5.6)</td>
<td>52.0 (15.0)</td>
<td>67.7 (3.8)</td>
</tr>
<tr>
<td>53.5</td>
<td>61.0 (4.7)</td>
<td>57.0 (2.6)</td>
<td>62.0 (9.9)</td>
</tr>
<tr>
<td>75</td>
<td>62.0 (2.6)</td>
<td>20.0 (10.0)</td>
<td>41.0 (14.0)</td>
</tr>
</tbody>
</table>

All values are given as mean with standard deviation in brackets. T1 - Before US application; T2 - Immediately after US application; T3 - 30 min later

Table 2: Relative hemoglobin measurements in venous leg ulcers and periulcer skin at 2 mm depth and 8 mm depth

<table>
<thead>
<tr>
<th>Frequency (kHz)</th>
<th>Depth (mm)</th>
<th>T1</th>
<th>T2</th>
<th>T3</th>
</tr>
</thead>
<tbody>
<tr>
<td>Ulcer</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>34</td>
<td>2</td>
<td>84.0 (1.4)</td>
<td>76.0 (9.9)</td>
<td>79 (2.2)</td>
</tr>
<tr>
<td></td>
<td>8</td>
<td>65.0 (5.5)</td>
<td>70.3 (14.6)</td>
<td>65.8 (13.0)</td>
</tr>
<tr>
<td>53.5</td>
<td>2</td>
<td>67.7 (22.8)</td>
<td>67.0 (14.4)</td>
<td>43.0 (3.5)</td>
</tr>
<tr>
<td></td>
<td>8</td>
<td>51.0 (2.8)</td>
<td>50.7 (5.5)</td>
<td>48.5 (5.1)</td>
</tr>
<tr>
<td>75</td>
<td>2</td>
<td>78.0 (7.8)</td>
<td>72.0 (8.2)</td>
<td>80.0 (7.8)</td>
</tr>
<tr>
<td></td>
<td>8</td>
<td>61.7 (12.5)</td>
<td>54.0 (5.3)</td>
<td>54.7 (8.5)</td>
</tr>
<tr>
<td>Periculcer skin</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>34</td>
<td>2</td>
<td>54.0 (6.9)</td>
<td>74.0 (19.8)</td>
<td>51.0 (9.5)</td>
</tr>
<tr>
<td></td>
<td>8</td>
<td>63.7 (15.5)</td>
<td>70.3 (7.8)</td>
<td>68.2 (15.9)</td>
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<tr>
<td>53.5</td>
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<td>65.0 (7.1)</td>
<td>60.0 (6.5)</td>
</tr>
<tr>
<td></td>
<td>8</td>
<td>40.0 (1.4)</td>
<td>45.7 (7.6)</td>
<td>45.0 (9.9)</td>
</tr>
<tr>
<td>75</td>
<td>2</td>
<td>48.0 (5.1)</td>
<td>40.0 (9.9)</td>
<td>55.0 (7.1)</td>
</tr>
<tr>
<td></td>
<td>8</td>
<td>42.7 (14.2)</td>
<td>47.0 (9.9)</td>
<td>44.3 (7.7)</td>
</tr>
</tbody>
</table>

All values are given as mean with standard deviation in brackets. T1- Before US application; T2 - Immediately after US application; T3 - 30 min later

Discussion

The skin has a complex vascular system that is not only involved in nutrition of cutaneous cells and tissues but plays a central regulatory role in the homeostasis of body core temperature. Arterio-capillary-venous units (AVCU) are the smallest units of the terminal vascular bed.[16] Chronic venous insufficiency (CVI) is a common disease.[3] CVI is associated not only with functional and morphologic changes of larges venous vessels like venous hypertension, valve incompetence and venous reflux but microvessels as well.[17] In CVI changes in skin capillaries like loss of density are stage dependent.[18] In addition, capillary morphology can be impaired as well.[19] The presence of pericapillary fibrin cuffs in the dermis of the gaiter skin is associated with a risk of developing leg ulcers.[20] Additionally, a local imbalance in coagulation...
and fibrinolytic activity has been documented with deposits of von Willebrand factor, urokinase-derived plasminogen activator, and plasminogen activator inhibitor.\textsuperscript{[21]}

CVI is characterized by an increase of hemoglobin bound oxygen content in skin. Thus a lack of oxygen is unlikely to be the primary reason for the development of skin lesions in CVI.\textsuperscript{[22]} The major pathway of tissue breakdown leading to leg ulcers seems to be the activation of leukocytes and neutrophils with an ongoing inflammatory response.\textsuperscript{[4,5]}

Venous stasis causes an increase of the amount of blood in microcirculation. The rHb value may exceed 90 AU mainly due to venular blood volume. It reduces the amount of water in the vessels and therefore increases the edema. SO\textsubscript{2} as detected by micro lightguide technique reflects the situation in the nutritive microvessels only. In venous insufficiency SO\textsubscript{2} decreases during orthostasis to values of <10%.\textsuperscript{[15]} During orthostasis, vasomotion frequency in patients with chronic venous insufficiency decreased significantly whereas vasomotion frequency in control subjects increased significantly. The failure to increase vasomotion frequency in CVI corresponds to longer dilatation of the distal venous vessels per time unit and increase in hematocrit.\textsuperscript{[23]}

In the present study, all measurements were performed in a horizontal position of the leg to avoid the effect of orthostasis. Patients were sitting during treatment to allow subaqual US application. This minimizes an orthostatic influence. Nevertheless, we observed some patients with rHb >90 AU but all pre-treatment values of SO\textsubscript{2} were >50 %. Diabetic wounds with SO\textsubscript{2} levels ≤ 50 % do not heal spontaneous.\textsuperscript{[14]}

In previous studies, low-frequency US improved granulation in chronic leg ulcers when used repeatedly.\textsuperscript{[6-10]} Healing time was lowered and rate of complete closure was increased when low frequency US was added to standard therapy independent from the pathology of chronic ulceration.\textsuperscript{[24-26]}

Pulsed low-frequency US of 25-50 kHz has been used successfully for the debridement of chronic leg ulcers. The underlying mechanism is surface cavitation.\textsuperscript{[1,2]}

Continuous low-frequency US in this experimental setting was studied with particular emphasis frequency-dependent effects on microcirculation as measured by non-invasive techniques. US caused an increase in SO\textsubscript{2} within the ulcers. On the other hand, rHb and blood cell velocity were not affected by a single US application. Furthermore, microcirculation increases as shown by remission
spectroscopy [Figure 4 and Table 4]. We observed a better hemoglobin oxygenation due to US therapy within the leg ulcers (34 kHz).

The value of low-frequency US in conservative leg ulcer treatment has mostly been shown by its effect on wound debridement and decrease of wound area. This report, for the first time, demonstrates that low-frequency US exerts mild positive effects on disturbed microcirculation of chronic venous leg ulcers mainly due to a better blood oxygenation. Dermatofibrosis may diminish the beneficial effects of such a treatment. The treatment is well tolerated and can be performed on an outpatient basis. Since effects are temporary further studies are necessary to investigate the effect of treatment series. Since series are usually performed in clinical practice this might result in more pronounced effects.

References


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