Capillary blood cell velocity in periulcerous regions of the lower leg measured by laser Doppler anemometry

Markus Stücker, Christina Huntermann, Falk Georges Bechara, Klaus Hoffmann and Peter Altmeyer

Department of Dermatology and Allergology, Ruhr-University Bochum, Germany

Background: The capillary blood flow of the nailfold can be measured by means of modern non-invasive techniques like the videocapillary microscope in vivo. To quantify the capillary blood cell velocity, apart from the nailfold capillaries, we used a new technique, the so-called laser Doppler anemometry (LDA).

Objective: The present study investigated how far laser Doppler fluxmetry (LDF), transcutaneous partial pressure of oxygen (tcpO$_2$), and LDA are capable of quantifying differences of cutaneous microcirculation between patients with leg ulcers and a healthy control group. The effects of intravenous prostaglandin E1 and pentoxifylline were also investigated.

Patients and methods: Ten patients with venous leg ulcers and 10 patients with mixed venous/arterial ulcers were investigated with LDF, tcpO$_2$, and LDA before and after injection of prostaglandin E1 and pentoxifylline. We measured the resting capillary blood cell velocity (rCBV), the maximum hyperemia, and the time to peak capillary blood cell velocity (tpCBV) during hyperemia after 4 min of suprasystolic occlusion and compared them with the results of a control group of 20 patients.

Results: Laser Doppler flow was increased in all patients during resting period, whereas the tcpO$_2$ was significantly decreased. LDF did not show an extension of tpCBV during reactive hyperemia after suprasystolic occlusion compared to the control group (73.6 ± 31.1 s vs. 164.1 ± 52.5 s, $P = 0.003$). TcpO$_2$ revealed significantly decreased tpCBV in patients with venous and mixed venous/arterial ulcers (90.1 ± 61.7 s vs. 162.7 ± 65.5 s, $P < 0.0001$). LDA showed no significant differences between patients and control group ($P > 0.8$). After application of prostaglandin E1, LDA revealed a significant increase of erythrocyte velocity (0.5 ± 0.18 to 0.74 ± 0.28 mm/s [$P = 0.01$]), whereas pentoxifylline had no significant effect. Capillary density increased significantly after application of prostaglandin E1 (5.1 ± 2.7/mm$^2$ to 8.9 ± 3/mm$^2$ [$P = 0.001$]) and pentoxifylline (5.3 ± 1.8/mm$^2$ to 8 ± 2.1/mm$^2$ [$P = 0.006$]).

Conclusion: The LDA is an important additional investigation tool for cutaneous microcirculation.

Key words: microcirculation – leg ulcer – blood flow – laser Doppler – capillary venous

© Blackwell Munksgaard, 2004
Accepted for publication 6 February 2004

Arterial and venous ulcers and the periulcerous lesions show disturbances in cutaneous microcirculation (1, 2). Pharmacological treatment with prostaglandin E1 and pentoxifylline aims at an improvement of microcirculation (3–6).

Capillary blood flow can be measured by means of non-invasive capillary microscopy, mostly performed on the nailfolds (7). Thus pathological changes and therapy effects can be quantified. In general, capillary loops of the skin are perpendicularly oriented to the skin surface. Only in a few areas of the human body, the lips, the nipple or the nailfold, the capillary loops run parallel to the skin surface. However, there has been almost no technique available to measure skin blood flow in capillary loops located at a 90° angle to the skin surface, like the lower leg. Therefore, it was not possible to quantify the erythrocyte velocity in capillaries of periulcerous regions.

Now with the laser Doppler anemometry (LDA) a device is available to measure the capillary blood flow in human capillaries oriented perpendicular to the skin surface. The reproducibility of this method has already been documented (8, 9).
In the present study, the following problems are investigated:

1. Is the LDA capable of demonstrating differences in cutaneous microcirculation of the periulcerous regions of venous/arterial ulcers and normal skin?
2. Are the effects of prostaglandin E1 and pentoxifylline in periulcerous regions detectable by LDA?

Patients and Methods

Patients

We investigated 20 patients, 10 with venous ulcers (age: 62.1 ± 11.6 years, five females, five males), 10 with mixed venous/arterial ulcers (age: 78.2 ± 4.6 years, six females, four males). Seven of 10 patients with venous ulcers had a deep venous insufficiency, all patients showed an insufficiency of the longer saphenous vein (grade III or IV to Hach). Patients with mixed venous/arterial ulcers showed an ankle-arm index of 0.59 ± 0.07 (ankle artery pressure: 88.5 ± 10.7 mmHg). All patients had no signs of skin disease at place of measurement, apart from typical changes due to venous or arterial genesis.

Both groups were compared to a control group. The control group for venous ulcers consisted of three healthy males and seven females (age 64.1 ± 11.7 years). The control group for patients with peripheral arterial occlusive disease also consisted of three healthy males and seven females (age: 56.4 ± 9.7 years). Clinical examination and Doppler sonography revealed no chronic venous insufficiency or peripheral arterial occlusive disease in the control groups. Subjects suffering from skin diseases (psoriasis, eczema) were excluded. Taking of medicaments, which have an influence on hemorheology, thrombocyte aggregation, or vessel tonus was also an exclusion criterion.

Measurement techniques

Cutaneous microcirculation was investigated by laser Doppler fluxmetry (LDF) (DRT4, Moor-Instruments, Exminster, Great Britain), measuring the transcutaneous oxygen tension (tcpO2) (TCM3, Radiometer, Kopenhagen, Denmark), and LDA (LDA) (CAM1, KK-Technologie, Oxford, UK). The CAM 1 includes a laser source (1.5 mW Laser Diode, wavelength 780 nm), focused by a microscope objective lens to a spot size of approximately 10 μm diameter. This results in a very small sample volume, so that the velocity in capillaries of 9.8–32.1 μm diameter can be singled out. Using the capillarometry, capillaries per field of view were counted.

Test procedure

All subjects were investigated in a lying position after acclimatization for at least 20 min (room temperature: 22–24 °C). Before suprasystolic occlusion, all parameters in rest were investigated for 2 min (2–4 cm from the ulcers). After occlusion, parameters were again registered for 2 min. Following parameters were determined: resting capillary blood cell velocity (rCBV), time to peak capillary blood cell velocity (tpCBV) during hyperemia, and the maximum hyperemia. All measurements were digitally recorded.

Patients with venous ulcers were again investigated 15 min after application of 100 mg pentoxifylline (Trental®, Aventis Pharma, Bad Soden, Germany). Patients with peripheral arterial occlusive disease received 40 mg of prostaglandin E1 (Prostavasin®, Schwartz Pharma, Mannheim, Germany) and were also examined 15 min after infusion. Infusion time in all patients was 2 h.

Statistics

The values of the patient groups were compared to those of the control groups using Student’s t-test for unpaired samples. Differences between the values before and after infusion therapy were tested using Student’s t-test for paired samples (SPSS for Windows 11.0, SPSS, Chicago, IL, USA).

Results

LDF was significantly increased in both patient groups (venous ulcers: 31.7 ± 12.4 AU vs. mixed venous/arterial ulcers: 61.7 ± 46.3 AU) compared to the respective control group (15.8 ± 6.94/16.8 ± 6.4 AU [P ≤ 0.01]). TcpO2 was significantly decreased in both groups (9.5 ± 11.7 vs. 23.6 ± 13.46 mmHg, P = 0.004/6.8 ± 4.0 vs. 18.7 ± 6.3 mmHg, P ≤ 0.001). Erythrocyte velocity was 0.5 ± 0.2 mm/s in all groups. Time to maximum hyperemia after suprasystolic occlusion was not extended compared to the control group by LDF (P > 0.05). TcpO2 was significantly decreased in patients with venous ulcers (73.6 ± 31.1 vs. 164.1
Before drug application, the LDA showed no significant differences between patients and control group (\(P > 0.8\)). Fifteen minutes after application, there was no difference detected by LDF or tcpO2. In contrast, the LDA revealed a significant increase of erythrocyte velocity after application of prostaglandin E1 (from 0.5 ± 0.18 mm/s to 0.74 ± 0.28 mm/s [\(P = 0.01\)]).

Capillary density raised from 5.1 ± 2.7/mm² to 8.9 ± 3/mm² (\(P = 0.001\)) after application of prostaglandin E1, and from 5.3 ± 1.8/mm² to 8 ± 2.1/mm² (\(P = 0.006\)) after medication with pentoxifylline (Figs 1–3).

**Discussion**

The present study investigated the cutaneous microcirculation of venous leg ulcers and mixed venous/arterial ulcers. We used three different measurement techniques: the LDF, the tcpO2, and the LDA.

The LDA offers a combination of video capillaroscopy and laser Doppler measurement. The system includes a laser source (1.5 mW Laser Diode, wavelength 780 nm), focused by a microscope objective lens to a spot size of approximately 10 \(\mu\)m diameter. This results in a very small sample volume, so that the velocity in capillaries of 9.8–32.1 \(\mu\)m diameter can be singled out. By this, almost every blood cell velocity is measurable, and for the first time a device is available to measure the resting capillary blood cell velocity in human capillaries oriented perpendicular to the skin surface. The instrument can be placed on almost all parts of the body (8, 9).

Before drug application, the LDA showed no significant differences between patients and control group (\(P > 0.8\)). Fifteen minutes after application, there was no difference detected by LDF or tcpO2. In contrast, the LDA revealed a significant increase of erythrocyte velocity after application of prostaglandin E1 (from 0.5 ± 0.18 to 0.74 ± 0.28 mm/s [\(P = 0.01\)]). No significant changes were demonstrated after application of pentoxifylline (increase from 0.53 ± 0.52 to 0.66 ± 0.8 mm/s [\(P = 0.29\)]). Capillary density raised from 5.1 ± 2.7/mm² to 8.9 ± 3/mm² (\(P = 0.001\)) after application of prostaglandin E1, and from 5.3 ± 1.8/mm² to 8 ± 2.1/mm² (\(P = 0.006\)) after medication with pentoxifylline (Figs 1–3).

Fig. 1. Resting capillary blood cell velocity (rCBV) before and after application of prostaglandin E1 measured by LDA. Significant increase of rCBV (0.5 ± 0.18 mm/s to 0.74 ± 0.28 mm/s [\(P = 0.01\)]).

Fig. 2. Capillary density (capillaries/mm²) before and after application of prostaglandin E1 in mixed venous/arterial ulcers. Significant increase of capillary density (5.1 ± 2.7/mm² to 8.9 ± 3/mm² [\(P = 0.001\)]).

Fig. 3. Capillary density before and after application of pentoxifylline in venous leg ulcers. Significant increase of capillary density (5.3 ± 1.8/mm² to 8 ± 2.1/mm² [\(P = 0.006\)]).
rates of tcpO₂ in rest. In isolated peripheral arterial occlusive disease, a lower tcpO₂ combined with a prolongation of time to maximum hyperemia is expected (2).

However, the LDA could demonstrate an increase of erythrocyte velocity and capillary density (capillaries/mm²) after application of prostaglandin E1. The capillary density was also increased after application of pentoxifylline.

The effect of both hemorheological could only be shown by LDA, whereas the LDF and tcpO₂ did not reveal any significant changes in cutaneous microcirculation of leg ulcers. In contrast, the effects of prostaglandin E1 and pentoxifylline on venous and arterial ulcers have been previously described (3, 15–8).

In summary, the LDA is an important additional technique for the evaluation of cutaneous microcirculation. In contrast to investigations of capillary blood cell velocity on the nailfold, the LDA allows to analyze all capillaries oriented perpendicular to skin surface.

References


Address:
Priv.-Doz. Dr. M. Stücker
Department of Dermatology and Allergology
Ruhr-University Bochum
Gudrunstr. 56
44791 Bochum
Germany
Tel: +49-234-509-3448
Fax: +49-234-509-3409
e-mail: M.Stuecker@derma.de