Vascular and Stromal Features in the Skin of the Lower Limb in Patients with Chronic Critical Limb Ischaemia (CLI) and Oedema

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Objective: peripheral oedema is often observed in limbs affected by chronic critical limb ischaemia (CLI) and is mainly subcutaneous in distribution. Previous work has shown that capillary filtration coefficient (CFC) in limbs with CLI and oedema was twice as great as that in the contralateral limb. These changes might be due to morphological changes. Transmission electron microscopy (TEM) was used to examine the morphological features of the capillary walls and surrounding stromal tissues in the skin of these limbs.

Material and methods: eight patients with unilateral CLI and peripheral pitting oedema (four men, four women, a mean age of 81 ± 6.9 years) was studied. Skin biopsies were taken from the pulp of the first toe, interdigital space between the first and second digits and dorsal part of forefoot just prior to amputation.

Results: stromal oedema and dilated capillaries were most prominent in the distal part of the foot. Some of the capillaries were filled with blood cells and some were empty. The endothelium of the dilated vessels was elongated and distended. In some patients a number of capillaries were collapsed with degenerate endothelial cells. “Gaps”, i.e. large openings, were found between the elongated oedematous endothelial cells. The basal lamina was thickened in all patients. Stromal haemorrhage and degeneration were seen in approximately 50% of patients.

Conclusion: CLI causes ultrastructural changes in the capillary endothelium and surrounding stroma. The presence of large gaps between endothelial cells as well as an increased capillary pressure may enhance transcapillary transudation, and are most likely the causative factors in the formation of the ischaemic oedema. The stromal haemorrhage as well as degeneration probably signifies a terminal stage of CLI.

Key Words: Critical limb ischaemia; Oedema; Capillaries; Endothelium; Stroma; Transmission electron microscopy.

Introduction

Patients with chronic critical limb ischaemia (CLI) have a poor outcome in terms of survival and limb salvage.1,2 The majority show signs of lower limb oedema, which is not caused by deep venous thrombosis (DVT)3 and which is mainly localised in subcutaneous tissue of the ankle and foot.4 A recent study revealed that capillary filtration coefficient (CFC), a measure of capillary permeability, in limbs with CLI and oedema was twice as great as in the contralateral limb.5 These changes in the capillary wall permeability might be due to disruption of endothelium causing an increased net transcapillary filtration rate, leading to oedema formation.5,6 Oedema increases the diffusion distance between capillaries and stromal cells which may lead to a further increase of tissue hypoxia in an already severely ischaemic limb.7,8 Therefore, it is relevant to further investigate the pathogenesis of oedema formation in limbs with CLI. Much research has been done on the quantitative aspects of the ultrastructure of capillaries from a number of animal and human tissues, including morphometric analysis after inducing an acute (<3 h) ischaemia.9 However corresponding data from chronically ischaemic human tissues are scarce. The purpose of the present study was to apply transmission electron microscopy (TEM) to examine the morphological features of the capillary walls and surrounding stromal tissues in the skin of patients with CLI and oedema.

Patients

CLI was defined according to the Second European Consensus Document on CLI (1991).10 Eight patients with unilateral CLI and peripheral
The skin specimens were traumatised as little as possible, and immediately put in a fixative for electron microscopy.

**Transmission electron microscopy (TEM)**

After fixation in a phosphate buffered solution of 1% glutaraldehyde and 4% formaldehyde, the skin samples were postfixed in 1% osmium tetroxide, dehydrated in graded alcohol and embedded in Epon. Between 10 and 30 small tissue pieces were embedded from each specimen. Semi-thin sections were cut with a glass knife, stained with toluidine blue and used for light microscopic orientation. Representative areas were selected. Ultra-thin sections were cut with a diamond knife, placed on copper grids and contrasted with uranyl acetate and lead citrate. The sections were then examined under TEM (Phillips CM 10).

**Results**

Details of the electron microscopic examination are presented in Table 3. The following characteristic features were found in all cases:

- Stromal oedema was most dominant at the distal part of the foot.
- Dilated capillaries were seen with increasing number at the distal part of the foot. Some of them were filled with blood cells and some were empty (Figs 1 and 2).
- Dilated smaller arteries without any proliferation or hypertrophy of smooth muscle cells layer in the wall of these arteries were observed in all patients and in all biopsy locations.
- The endothelial layer of the dilated vessels was elongated and distended. At the pulp of the first toe and at the level of the first interdigital space we observed an increasing number of elongated endothelial cells. In some patients a number of capillaries were collapsed with degenerated endothelial cells (Figs 3 and 4).
- "Gaps", i.e. large openings, were found between the elongated/extended oedematous endothelial cells. In some patients at site one or two, many erythrocytes were seen passing through these larger gaps, indicating bleeding into stroma. The signs of stromal haemorrhage were seen in approximately 50% of patients (Fig. 5).
- The basal lamina was multiplicated with a considerably increased thickness seen in cross sections of capillaries from all patients (Figs 6 and 8).

**Methods**

To classify the patients as having CLI, ankle and brachial pressures were measured with ultrasound Doppler technique. In the limbs with CLI where Doppler signals were not detectable, ankle pressure was defined at 15 mmHg.

**Biopsies**

Biopsies of the skin including part of the subcutaneous tissue of approximately 1 cm in diameter were taken from the following areas: (1) pulp of the first toe, (2) interdigital space between the first and second digits, (3) dorsal part of forefoot. The biopsies were performed after administration of epidural anaesthesia, just prior to the amputation, without the use of local anaesthesia.
Table 2. Transmission electron microscope features of capillaries and stroma in the foot skin of patients with CLI and oedema. Biopsies of the skin were taken from 3 different sites, 1: Pulp of the first toe, 2: Interdigital space between the first and second digits and 3: Dorsal part of the forefoot.

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**Capillaries**
- Dilated capillaries
  - Patients I, II, III, IV, V, VI, VII, VIII
  - Sites 1, 2, 3
- Extended endothelium
  - Patients I, II, III, IV, V, VI, VII, VIII
  - Sites 1, 2, 3
- Gaps in endothelial layer
  - Patients I, II, III, IV, V, VI, VII, VIII
  - Sites 1, 2, 3
- Thickness of external lamina
  - Patients I, II, III, IV, V, VI, VII, VIII
  - Sites 1, 2, 3
- Granulocytes in capillary lumen
  - Patients I, II, III, IV, V, VI, VII, VIII
  - Sites 1, 2, 3

**Stroma**
- Degeneration
  - Patients I, II, III, IV, V, VI, VII, VIII
  - Sites 1, 2, 3
- Haemorrhage
  - Patients I, II, III, IV, V, VI, VII, VIII
  - Sites 1, 2, 3
- Oedema
  - Patients I, II, III, IV, V, VI, VII, VIII
  - Sites 1, 2, 3
- Granulocytes
  - Patients I, II, III, IV, V, VI, VII, VIII
  - Sites 1, 2, 3
- Mast cells
  - Patients I, II, III, IV, V, VI, VII, VIII
  - Sites 1, 2, 3

- Absent, + Pronounced, ++ More pronounced, * Material not available.
Fig. 1. An example of a considerably dilated and relatively “empty” capillary with extended endothelial cells. No thickening of basal lamina. A granulocyte is present in lumen (uranyl acetate (UA)/lead citrate (LC) × 2200).

Fig. 2. A dilated capillary with abundant blood cells in lumen (UA/LC × 1200).

Fig. 3. Area with degeneration and a capillary. Note gaps in endothelial layer (arrow) (UA/LC × 2900).

Fig. 4. Remnant of blood cells in a capillary lumen. A thickened basal lamina is still present. Degeneration is also present in stroma (UA/LC × 2900).

Fig. 5. Degenerated stroma with haemorrhage as indicated by the abundance of red blood cells (RBC) (UA/LC × 2900).
Fig. 6. Capillary with multiplicated basal lamina surrounding endothelial cells (UA/LC × 4900). Higher magnification of another capillary wall with thickening of basal lamina (UA/LC × 6600).

Fig. 7. A capillary with a granulocyte in lumen. A mast cell (arrow) is present in stroma (UA/LC × 2200).

Fig. 8. Two capillaries filled with destructed blood cells. One of the vessels is surrounded by a normal basal lamina, the other is thickened (arrows) (UA/LC × 2900).

in the skin of the distal part of the foot. Since the ischaemia is usually most pronounced in the distal part of the limb, this oedema is probably related to the severity of ischaemia.

The finding of dilated capillaries in all investigated areas probably indicates an increased hydrostatic pressure in the capillaries (Pc) which has been sustained for a period of time in chronically ischaemic limbs. The increased Pc is presumably the result of arteriolar dilatation due to local hypoxaemia, which is a very strong vasodilating stimulus. Additional factors may contribute to increasing capillary pressure, for instance an abolished veno–arteriolar response (VAR). VAR is a sympathetic axon reflex which causes arteriolar constriction in the dependent foot. This reflex is probably abolished in the critically ischaemic foot with oedema. Therefore, lowering of the limbs to relief rest pain may lead to arteriolar dilatation. Furthermore, lowering of the ischaemic limb increases distal venous pressure as well. Both arteriolar dilatation and elevated venous pressure most likely lead to increased Pc. Increased Pc subsequently enhances transudation of fluid through the capillary wall and is probably one of the aetiological factors in the development of ischaemic oedema.

Another factor to explain the finding of dilated capillaries could be a loss of contractile tone of the endothelial cells as a result of hypoxaemia. Previous studies have demonstrated that capillaries lose their tone on oxygen deprivation.
This study also demonstrated dilatation of precapillary arteries. This is probably an expression of a homeostatic function, where vasodilatation is caused by a "myogenic response" to low blood perfusion and a "metabolic response" to vasoactive substances due to local ischaemia.17

We found several morphological changes in the endothelial cells of the dilated capillaries. Volume expanded endothelial cells were most frequently seen at the proximal sites of the foot. The finding of collapsed and degenerated endothelial cells were more pronounced distally in the foot. These morphological changes of the endothelium represents different stages of cellular injury, indicating that the most severe cell damages mostly occurred in the distal parts of the foot. In addition, there were areas where the continuity between the elongated and degenerated endothelial cells was disrupted by relatively large openings (gaps). These gaps seem to cause an increased permeability, thus promoting the development of ischaemic oedema, and may explain the increased capillary filtration coefficient (CFC), which we previously found in patients with CLI and oedema.5

Some of these gaps were sufficiently large to allow passage of blood cells, resulting in stromal haemorrhage. Endothelial and stromal degeneration as well as stromal haemorrhage were seen in 50% of patients in our study. These findings were mostly present in patients with extensive critical ischaemia, who ended up with a primary femur amputation.

A thickened multiplicated basal lamina surrounding dilated capillaries was observed in all investigated areas. This layer may represent a compensatory mechanism to prevent further dilatation of these capillaries, which have been exposed to an increase Pc over time. The stromal haemorrhage as well as degeneration of large gaps between endothelial cells was disrupted by relatively large openings (gaps). These gaps seem to cause an increased permeability, thus promoting the development of ischaemic oedema, and may explain the increased capillary filtration coefficient (CFC), which we previously found in patients with CLI and oedema.5

A substantial number of dilated capillaries were emptied for erythrocytes. Passage through capillaries may be restricted due to a reduced deformability of the erythrocytes and leukocytes as a consequence of reduced blood flow and shear stress.10 Some previous studies have postulated that external compression by oedema formation might prevent erythrocytes from entering the nutritional capillaries.5,9,19 The present study does not support such a view, since we found dilated capillaries in conjunction with stromal oedema.

The finding of an abundance of granulocytes both in stroma and the capillary lumen and absence of lymphocytes indicates an acute rather than a chronic inflammatory response in the microcirculation. Tissue damage from ischaemia is not only due to hypoxia per se but also to an inflammatory reaction in which activated leukocytes and platelets may play a role in causing an increased capillary permeability and thereby oedema formation.2622 Although previous research has addressed intracapillary leukocyte "blockage" following acute ischaemia,22,25 this phenomenon was not observed in the present study.

Mast cells were frequently present in the stroma. The mast cell granules, containing histamine, heparin and various enzymes, are released by chemical factors early in the inflammatory reaction.24 The releasing of histamine type mediators have vasodilatory effect 25 and may induce gaps between the endothelial cells.26 However, the relatively large gaps exposing parts of the basal lamina that was found in the present study are more likely a result of endothelial cell degeneration.26

Conclusion

The present study showed that chronic ischaemia in the lower limb may cause hypoxic damage of the capillary endothelium and surrounding stroma. The presence of large gaps between endothelial cells as well as an increased capillary pressure seem to enhance the transcapillary transudation, and are most likely the causative factors in the formation of the ischaemic oedema. The oedema does not seem to compromise the microcirculation by compressing capillaries, but rather by increasing the oxygen diffusion distance. The stromal haemorrhage as well as degeneration probably signifies a terminal stage of CLI.

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