In vivo Microscopic Study of Microcirculatory Perfusion of the Skin of the Foot in Peripheral Vascular Disease

M. Lamah*, P. S. Mortimer and J. A. Dormandy

Departments of Vascular Surgery and Dermatology, St George’s Hospital Medical School, London, U.K.

Objectives: The aim of this study was to determine the proportion of perfused capillaries in the skin of the foot in patients with peripheral vascular disease, and compare it with that in normal subjects.

Design: Experimental study comparing capillary perfusion in nine patients with severe peripheral vascular disease (group 2) with seven age- and sex-matched control subjects (group 1).

Materials and methods: Using in vivo video microscopy, a method was developed to measure the ratio of perfused to total capillaries, by comparing the numbers of corresponding capillaries before and after intravenous injection of sodium fluorescein.

Results: The mean percentage ratio of perfused to total capillaries was 54.7% (range 41–87%, standard deviation 16.5) in group 1, and 86.0% (range 62–100%, standard deviation 13.2) in group 2 (p<0.001, t-test).

Conclusion: A significantly higher proportion of capillaries is perfused in the skin of the foot of patients with severe peripheral vascular disease than in that of normal subjects. This is of important pathophysiological significance and may have clinical implications with regard to the role of pharmacological intervention in severe limb ischaemia.

Key Words: Capillary perfusion; Microscopy; Peripheral vascular disease.

Introduction

Developments in the measurement of microcirculatory function over the last two decades have highlighted the role of abnormal microcirculatory behaviour in arterial disease. Anatomical findings of reduced capillary density and abnormal capillary morphology, as well as abnormalities in capillary perfusion dynamics, have been reported in patients with peripheral vascular disease. The aim of this study was to compare the ratio of perfused to total capillaries in the skin of the dorsum of the foot in healthy subjects and in patients with severe peripheral vascular disease causing ischaemic ulceration.

Subjects and Methods

Subjects

Seven control subjects (group 1: 3 male, 4 female, mean age 73 years, range 44–79 years, 3 smokers) and nine patients (group 2: 4 male, 5 female, mean age 74 years, range 59–87 years, one smoker) with severe peripheral vascular disease (mean ankle-to-brachial pressure index 0.35, range 0.1–0.45, and all with ischaemic ulceration of the dorsum of foot), were studied. The control subjects were all inpatients awaiting minor surgery, and had no history of any skin, cardiovascular or peripheral vascular disease, their mean ankle-to-brachial pressure index being 0.94. Ethical approval was given by the Local Ethics Committee at St George’s Healthcare NHS Trust, and informed consent obtained from every subject.

Intra-vital microscopy

Microscopy of skin capillaries was performed using a Wild–Leitz epi-illuminated microscope, linked to a video camera (Hitachi CCTV KP-161). Microscopic images were thus passed onto a screen (Hitachi VM 921 K) for on-line monitoring, and simultaneously stored on videotape. Subsequently, still-frame video prints were produced by playing back the tape in a separate video recorder linked to a video printer (Sony Multiscan UP 930). All data analysis was made retrospectively off-line from the prints. Capillary density

* Please address all correspondence to: M. Lamah, 10 Langley Road, London SW19 3NZ, England, U.K.
was calculated by dividing the capillary count by the area of interest.

**Protocol**

The subject sat on a dental chair, in a room kept at constant temperature (22–24 °C), with both feet at 45° dependency. The leg under study was immobilised in a vacuum pillow, and skin temperature of the foot was monitored by a skin thermistor (YSI Tele-thermometers). A thin film of glycerin oil was applied to the skin to reduce light reflection and increase the transparency of the skin. Each subject was allowed to acclimatise for 10 minutes and videomicroscopy was then performed using the 2.5× objective (giving a final magnification of exactly 40-fold), at near-maximal light intensity. The objective was positioned perpendicularly to the skin surface, the picture focused and finally recorded on videotape. This method has been developed and its reproducibility tested in a separate study by the authors.  

The proportion of perfused to total capillaries was determined in the following way: a random area on the dorsum of foot (away from the ulcer in patients) was marked with a “microtattoo”, and native microscopy of that area performed. A 20% solution of sodium fluorescein (0.3 ml per litre of estimated blood volume) was then injected intravenously and, by reference to the microtattoo, the same area of skin was microscoped for a minute. At the time of the second measurement, exactly the same capillaries were re-microscoped, thus eliminating any differences in capillary counts that may arise as a result of spatial heterogeneity of capillary density which exists even between immediately adjacent areas of skin. The two images, representing the “total” (before injection) and the “perfused” (1 minute after injection) capillary beds were subsequently compared and the ratio calculated.

**Discussion**

The haemodynamic disturbance which results from atherosclerotic disease of the large arteries is undoubtedly the fundamental process in the pathogenesis of tissue ischaemia in patients with peripheral vascular disease. However, in recent years it has generally become accepted that other systems are also disturbed and that the overall pathophysiology of limb ischaemia represents a multifactorial process involving a complex interaction between macrocirculatory, microcirculatory, haemorrhheological and biochemical factors. In relation particularly to the microcirculation, several workers have reported abnormalities in capillary perfusion dynamics in these patients. These include attenuated physiological autoregulatory mechanisms, a lower prevalence of flow motion activity, and inhomogeneity of microvascular perfusion in ischaemic areas.

To our knowledge, there are no published data comparing the ratio of perfused to total capillaries in the skin of healthy subjects with that in patients with peripheral vascular disease. This study uses a method in which exactly the same capillaries are re-imaged after the injection of fluorescein, thus eliminating any sampling error and giving a precise ratio of the number of perfused to total capillaries.

We have shown that a significantly higher proportion of nutritional capillaries of the skin are perfused in patients with peripheral vascular disease than in healthy subjects. This may be an important mechanism for increasing oxygen and nutrient supply to ischaemic skin. However, the observation of fluorescence in a capillary gives no indication of the volume per unit time of blood flow in that capillary. It could be therefore that, even though relatively more capillaries contain flowing blood, the total volume of blood flow...
per unit volume of nutritional capillaries in a given area is less than that in controls. The only certain conclusion to be drawn from this study is that a significantly higher proportion of capillaries are open to blood flow in peripheral vascular disease.

The mechanisms underlying this phenomenon can only be speculated about, and may include reduced tissue oxygen tension and increased local concentration of metabolites, which are well known to stimulate local arteriolar vasodilatation. Irrespective of the mechanisms, the findings in this study may have some implications regarding pharmacological attempts to increase blood flow in these patients. Since skin nutritional capillaries are already "open" to a near-maximal extent, further recruitment of capillaries by pharmacological manipulation may have a relatively minor effect on the total number of open capillaries and, therefore, on the overall perfusion of the tissue.

References
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Accepted 25 January 1999