Review

Assessment of endothelial and neurovascular function in human skin microcirculation

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Peripheral microvascular dysfunction has been described in many physiological and pathological conditions. Owing to its accessibility, the cutaneous microcirculation provides a unique index of microvascular function. Skin microvascular function has therefore been proposed as a prognostic marker or for evaluating the effect of drugs on the microcirculation. Various reactivity tests, coupled with techniques measuring skin blood flux, are used to non-invasively explore both endothelial and neurovascular microvascular functioning in humans. We review the advantages and limitations of the main reactivity tests, including post-occlusive reactive hyperemia, local thermal hyperemia, pressure-induced vasodilation, and iontophoresis of vasodilators, combined with measurement techniques such as laser Doppler and laser speckle contrast imaging. Recent advances in our comprehension of the physiological pathways underlying these reactivity tests, as well as technological developments in microcirculation imaging, have provided reliable and reproducible tools for studying the microcirculation.

The microcirculation and its regulation

The microcirculation refers to arteries with the smallest resistance (less than ~150 μm in diameter), arterioles, capillaries, and venules. Whereas the capillary network is essential for nutrient and gas exchanges between blood and tissue, arterioles are prominently implicated in blood flow regulation [1]. The mechanisms involved include arteriolar myogenic response [2], flow-induced vasodilation (in response to wall shear stress), and metabolic and neural control. Among the latter, sympathetic activation usually causes vasoconstriction, whereas other neurons release vasodilators such as calcitonin gene-related peptide (CGRP) and substance P.

At the cellular level, vascular smooth muscle cells (SMCs) are common effectors. They closely interact with the endothelium, which releases mediators involved in relaxation and contraction. Endothelium-dependent relaxation involves different pathways. Nitric oxide (NO) is a potent vasodilator generated by NO synthase (NOS) that diffuses to adjacent SMCs. The NO pathway interacts with other regulatory mechanisms. Reactive oxygen species scavenge NO and decrease its production through NOS uncoupling [3]. By contrast, CGRP (which also induces vasodilation through direct action on SMCs) stimulates NO release [4], whereas NO has the capacity to inhibit sympathetic vasoconstriction through postsynaptic mechanisms [5].

Eicosanoids comprise a number of key endothelial vasodilators such as prostacyclin (PGI_2) and cytochrome P450 (CYP) metabolites, namely epoxyeicosatrienoic acids (EETs), which account for the endothelium-derived hyperpolarizing factor (EDHF) pathway [6].

Microvascular dysfunction in cardiovascular disease

Microvascular dysfunction has been described in both physiological (aging) and pathological conditions, with special relevance in cardiovascular disease [3,7]. Endothelial dysfunction and arterial remodeling/rarefaction have

Glossary

Arterioles: small-diameter vessels (usually <100 μm) located downstream of feed arteries. They have a smooth muscle layer conferring their ability to change lumen diameter.
Arteriolar myogenic response: perfusion pressure has a key role in the local regulation of arteriolar blood flow. Increased intraluminal pressure induces vasoconstriction, whereas decreased pressure leads to vasodilation. This phenomenon, known as the arteriolar myogenic response, maintains microvascular perfusion despite fluctuations in arterial pressure.
Capillaries: dense vascular network made of parallel branches downstream of terminal arterioles. They are endothelial tubes surrounded by a membrane and pericytes.
Dermis: layer of the skin located between the epidermis and subcutaneous tissue. It is approximately 1.5–5 mm thick and contains blood vessels and nerve endings. Capillaries and the vascular plexus located in the upper papillary dermis represent the most important part of the skin microcirculation.
Epidermis: multilayered region comprising the outermost layers of the skin. There are no blood vessels in the epidermis.
Iontophoresis: method for transdermal drug delivery based on the transfer of charged molecules using a low-intensity electric current. It is non-invasive and has several advantages compared to passive transdermal administration, such as faster drug release and better control of the dose delivered. Factors involved in iontophoretic transfer include the concentration and the size of the molecule, the proportion ionized, the intensity of the current, whether it is continuous or discontinuous, and its duration. The nature of the skin surface (thickness, glabrous or not) and its integrity also play a key role.
Venules: located downstream the capillary network. Their organization parallels that of arterioles. However, smooth muscle is absent in the smallest venules.

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been observed in animal models and in patients with hypertension [7–9]. Although part of this impairment is the consequence of elevated blood pressure, recent evidence suggests that increased reactive oxygen species (ROS) in the endothelium contribute to the pathophysiology of hypertension by limiting the bioavailability of NO [7,10,11].

Microvascular complications of diabetes have been related to endothelium-dependent [12] and -independent dysfunction [13]. Again, the cause and effect relationship is not clear because microvascular dysfunction may participate in the pathogenesis of diabetes [7,14]. A recent meta-analysis of prospective clinical studies showed that microvascular dysfunction is associated with a higher incidence of type 2 diabetes, strengthening this hypothesis [15]. Proposed mechanisms are impaired capillary recruitment, which would increase insulin resistance by limiting uptake of glucose from the blood [7,15], and apoptosis of β cells due to pancreatic microvascular endothelial dysfunction [15].

These examples highlight the potential relevance of early detection of microvascular dysfunction [16]. However, in most cases direct study of the target microvascular bed is technically challenging (e.g., the coronary microcirculation), stressing the need for appropriate surrogate markers.

The skin microcirculation as a model of generalized microvascular endothelial and neurovascular function

The cutaneous microcirculation has anatomical and physiological specificities to fulfill its thermoregulatory function. It is organized as two horizontal plexuses in the dermis: the upper network (from which capillary loops arise) located in the papillary dermis is connected to a lower dermal–hypodermal network through ascending arterioles and descending venules [17]. There are anatomical differences according to the region. Arteriovenous anastomoses that bypass the capillary circulation [1] are found in glabrous skin (especially the digits), and the density of capillary loops and ascending arterioles is heterogeneous between vascular beds [17]. Moreover, the skin presents a high density of nerve fibers compared to other tissues, which explains the major influence of neural control on skin microvascular reactivity. Conversely, autoregulation through arteriolar myogenic response is rather low [14].

Owing to its accessibility, the cutaneous microcirculation has been suggested as a model of generalized microvascular function [18]. However, this implies an association between microvascular dysfunction in the skin and in other vascular beds. In the past 10 years, it has been shown that skin microvascular reactivity is abnormal in a variety of cardiovascular diseases. For example, coronary disease correlates with cutaneous endothelium-dependent microvascular reactivity [19]. In the same way, skin microvascular function is an independent marker of cardiovascular disease in patients with type 2 diabetes [20] or end-stage kidney disease [21].

To summarize, the skin circulation is an accessible vascular bed for which dysfunction correlates with markers of cardiovascular disease. The assessment of skin microvascular function is therefore of great interest to provide insights into mechanisms underlying disease. It could also be a promising prognostic marker or be useful in evaluating the effect of drugs in cardiovascular disease (Figure 1).

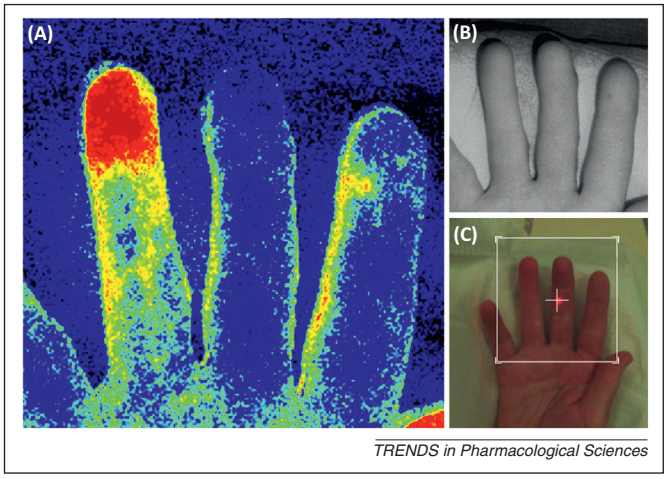

Figure 1. **(A)** Measurement of skin perfusion assessed by laser speckle contrast imaging 30 min after iontophoresis of a vasodilator on the ring finger and sodium chloride on the middle finger in a patient with secondary Raynaud's phenomenon. Colors range from dark blue (no perfusion) to red (high perfusion). **(B)** Intensity image representing backscattered light. **(C)** Region of interest (i.e., scanned area).

Non-invasive methods to assess skin microvascular function

A variety of non-invasive techniques have been developed to explore the skin microcirculation. Videocapillaroscopy consists of direct observation of skin capillaries with a microscope. It is routinely used on the nailfold, where capillaries are parallel to the skin surface, in the diagnosis of systemic sclerosis (Box 1). Outside the periungueal region, where capillaries are perpendicular to the skin surface, videocapillaroscopy can be used to assess functional capillary density and capillary recruitment [7,22]. Other techniques such as orthogonal polarization spectral imaging and sidestream dark-field imaging provide images of the microcirculation with a high level of contrast on organs covered by a thin epithelial layer [23]. To date, they have

Box 1. Exploration of skin microcirculation in Raynaud's phenomenon

Skin microvascular function can be explored as a representative vascular bed for the study of mechanisms underlying cardiovascular disease (see the text for details). In other diseases such as Raynaud's phenomenon (RP), however, the cutaneous microcirculation is specifically impaired. Abnormal microvascular function has been observed in patients with primary RP [68], and both functional and structural abnormalities are a hallmark of RP secondary to systemic sclerosis [85,86]. Nailfold videcapillaroscopy is a non-invasive technique that has been used for decades to evaluate capillary density and morphology. It is used routinely in the early diagnosis of scleroderma spectrum disorders [87,88] and has also been proposed as a prognostic marker [89]. Measurement of digital skin perfusion with laser Doppler correlates with microvascular abnormalities in systemic sclerosis patients [90,91]. In the past few years, this technique has been widely used in studies to assess the effect of drugs in RP [69,70,92–94]. These techniques have also been used in other situations in which the skin microvasculature is specifically impaired or damaged, such as diabetic ulcerations [95], burns [96,97], flaps [98], and wounds [99].