Vascular Responses in the Skin: an Accessible Model of Inflammation

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The skin is the most accessible organ of the human body in which to study the inflammatory process. Until recently, changes in vascular perfusion and permeability could only be superficially visualized. A novel combination of techniques has now provided the appropriate tools with which to study this more extensively in humans.

The responsiveness of the microvasculature of the skin to both physical and emotional stimuli has long been recognized. On the one hand, the Shakespearean poet, observing the emotional response of blushing and blanching, saw the skin as a mirror to the soul. On the other, the scientist, seeing similar responses more prosaically, thinks only of inflammation and temperature control! However, even for the scientist, the skin has been somewhat of an enigma, because for many years local assessment of the reaction of the human dermal vasculature to noxious stimuli, for example the weal-and-flare response, had to be performed from outside the skin surface. Thus we have been limited to observing changes in skin color and measuring skin temperature to gain information about dermal blood flow. The application of molecular technology to the study of these functional responses in relevant models, particularly in humans in vivo, has proved frustratingly difficult, because most require direct access to the dermal tissues while causing minimal disruption or damage to the local environment.

In an effort to overcome these problems, a number of groups have developed noninvasive techniques of imaging blood flux to quantify temporal and spatial variations in responses of the vasculature to dermal provocation. Used in combination with dermal microdialysis, a minimally traumatic technique that allows sampling of the extracellular compartment of the dermis, it has become possible to explore the mediator mechanisms underlying vascular control in the skin in ways that have hitherto not been possible.

Skin microcirculation

As a consequence of its role as a protective barrier and an important thermoregulatory organ, the skin has an extensive and well-developed microcirculation (2). It is organized as two horizontal plexuses: the upper, which lies 1–1.5 mm below the epidermis and supplies the microvasculature of the papillary dermis, and the lower, which is at the junction between the dermis and the underlying subcutaneous tissue. Blood flow to the skin in a thermoneutral environment ranges between 10 and 20 ml min⁻¹·100 g⁻¹, with a minimal flow of <1 ml min⁻¹·100 g⁻¹ and maximal flow at times of severe heat stress of 150–200 ml min⁻¹·100 g⁻¹. Control of this extremely labile vascular bed is varied, combining neural, humoral, and local influences (8).

The endothelium, nearly 30 m² of which lines the dermal vasculature, plays a pivotal role in maintaining vascular homeostasis. It is a source of a wide range of vasoactive autocoids with both autocrine and paracrine actions. It is also a functional barrier to the movement of water, solutes, and cells into the extravascular space. Many of the substances derived from the endothelial cells, as well as from the plasma and infiltrating leukocytes, have been implicated in the modulation of the inflammatory vascular response through their actions at the endothelial wall. It is the specific properties, biological activity, and interactions of these mediators that have been hypothesized to determine both the severity and duration of inflammatory responses. However, because of these interactions, it has proved difficult to investigate, and even more complex to model, the pathogenesis of inflammation in many tissues. We will argue that the skin represents an important and relevant model in which inflammatory processes may be elucidated.

Noninvasive imaging of blood flux

Because of its accessibility, it might be assumed that the quantitative assessment of microvascular perfusion should present few problems. However, although our knowledge of the ultrastructure and organization of the vascular plexus in human skin is considerable, the number and diversity of methods developed in an attempt to measure flow within the cutaneous circulation shows how problematic this can be (13). Current methods include measurement of transcutaneous oxygen, radionucleotide techniques, thermography, ultrasound, laser-Doppler fluximetry, and, most recently, optical imaging techniques. Among all of the methods, the laser-Doppler technique, which uses a measure of red blood cell movement to evaluate vascular flux, has proved one of the most suitable and best validated for use in a clinical setting to date (Fig. 1).

High-resolution scanning laser-Doppler imaging uses a low-power, ~2-mW, 633-nm, red He-Ne laser to scan the skin in a raster pattern and build up a two-dimensional image of red blood cell flux up to a depth of ~0.6–1 mm. It is able to provide a real-time output of temporal and spatial changes in skin blood flux, particularly during dermal provocation. Because the scanning head is mounted 30 cm or more above the skin surface, it also allows space for manipulation within the...
scanned area. Thus it has been used by many groups in combination with techniques to recover tissue mediators in their investigation of the cellular mechanisms underlying local vascular control (3).

**Recovery of metabolites by dermal microdialysis**

A number of sampling techniques have been developed for the recovery of substances from the tissue space. Microdialysis has the advantage over other dermal sampling techniques, such as that of needle aspirate, blister fluid, or tissue exudate, in that it causes minimal tissue trauma, is well tolerated, and can be used to follow the temporal variations in the generation and release of a substance at a discrete location within the tissue space. In combination with blood flux imaging, microdialysis affords a powerful tool with which to investigate the local vascular function (Fig. 2).

Microdialysis was originally developed to recover neurotransmitters from the brains of experimental animals. Subsequently, it has been modified to sample endogenous chemicals within the extravascular space in a number of organs in vivo, including human skin. It has also been used to follow the pharmacokinetics of a wide range of xenobiotics within the dermis. The endogenous substances recovered from the skin using microdialysis include metabolites such as glucose and lactate, vasoactive mediators such as histamine, nitric oxide, cyclic nucleotides, and eicosanoids, and, most recently, plasma proteins, cytokines, and neuropeptides (6). It is thus possible to identify and, if the recovery system is well characterized, quantify mediators involved in the development and resolution of dermal inflammation. This has considerable advantages over studies using pharmacological antagonists or inhibitors of the response, which only provide circumstantial evidence about involvement of the mediator under examination.

The recovery of a substance by microdialysis is governed by the same principles as that of exchange of small hydrophilic molecules across the microvascular wall, i.e., that of passive diffusion down a concentration gradient, as described by the Fick principle. The ability to recover a solute by microdialysis will thus be determined by the nature of the solute (its size, charge, and aqueous solubility) and the characteristics of the membrane (its area, porosity, and the rate at which it is perfused). Recovery may also depend on the nature of the surrounding tissue space, including its volume, state of hydration, and the extent to which molecules are able to diffuse through it. Ironically, it is these factors, together with the variability of cutaneous perfusion, that compromise our ability to quantitatively recover the molecules recovered by dialysis (1).

A number of types of microdialysis probe are currently available commercially for diverse applications. However, for dermal microdialysis, many laboratories have constructed their own linear flow membranes from hollow dialysis fibers, such as those extracted from renal dialysis or plasmapheresis capsules. The fibers have an external diameter of between 200 and 400 μm and a molecular mass cutoff ranging from 2 kDa, used to recover small endogenous molecules such as nitric oxide, histamine, and tissue metabolites, up to 3,000 kDa, for cytokines, neuropeptides, and plasma protein recovery. The efficiency with which these molecules are recovered from the tissue space ranges from >80% for nitric oxide to ~20% for...
Nitric oxide: does it have a role in the local inflammatory response?

Nitric oxide can be synthesized by a number of cell types, which in the skin include vascular endothelial cells, nerves, mast cells, fibroblasts, Langerhans’ cells, and keratinocytes. Thus it has been postulated that, over and above its role in regulating vascular tone, nitric oxide has both pro- and anti-inflammatory actions. Because of its proinflammatory effects, nitric oxide has been implicated in the pathogenesis of a wide range of human diseases, including those of the skin. As a consequence of this, characterization of the activity of this powerful signaling molecule may lead to the development of new therapies not only for the treatment of inflammatory disease in skin but also in other tissues.

Nitric oxide, formed enzymatically from L-arginine by intracellular nitric oxide synthases (NOS), can diffuse readily across cell membranes and within the tissue space. Although it has a half-life of only a few seconds under normal physiological conditions, much of it is rapidly converted to NO\textsuperscript{2−}. This can be readily assayed in tissue fluid as NO/NO\textsuperscript{2−} by using chemiluminescent or other colorimetric or amperometric techniques. Significant concentrations of NO/NO\textsuperscript{2−} (assayed by chemiluminescence) have been recovered in dialysate from the skin of more than 100 healthy volunteers. Concentrations have been found to range from 0.3 to 1 μM and to depend on factors such as age, tissue status, cigarette smoking history and, in women, menstrual cycle. In studies in which inflammatory agonists such as histamine, acetylcholine, bradykinin, and the neuropeptide substance P have been used to provoke acute local inflammatory responses in the skin, rapid (<2 min) increases in dialysate levels of nitric oxide (and cGMP, through which NO has its effects on vascular smooth muscle tone) occur (Fig. 3), which peak at values of up to 10 times higher than basal levels and which appear to be causally related to an increase in local blood flow (4).

Inhibition of NOS by analogs of L-arginine, such as \textit{N}G\textsuperscript{-}G-nitro-L-arginine methyl ester (L-NAME), also delivered via the dialysis probe, has been shown to result in a fall in both basal and agonist-induced increases both in the concentration of nitric oxide and in local blood flow. Significantly, as also demonstrated in other studies using systemically delivered inhibitors, L-NAME only partially inhibits the peripheral vasodilator response to many agonists, including acetylcholine, histamine, bradykinin, and substance P. This lends weight to the tenet that, at the level of the microcirculation, NO-independent pathways may play a significant role in modulating agonist-induced changes in vascular tone.

Nitric oxide can also play an integral role in the immune response in skin. Indeed, inhibition of the inducible NOS pathway has been shown to have therapeutic effects in allergic contact disease and psoriasis. It may also play a role in the regulation of leukocyte trafficking within the skin and thus be pivotal to the progression and resolution of the inflammatory state. There is also growing evidence that nitric oxide is involved in the skin response to ultraviolet B (UVB) irradiation. Recent experiments in which dialysate from UVB-exposed skin was assayed for nitric oxide confirmed that nitric oxide synthesis was prolonged and that it made a significant contribution to UVB-induced erythema monitored at the same time intervals using laser-Doppler fluximetry and imaging (10). It is these demonstrable roles for nitric oxide in the pathogenesis of skin disease that will fuel the transitional science necessary for the future therapeutic intervention.
**Leaky vessels: can we monitor them in humans?**

Under normal conditions, the endothelium acts as a relatively impermeable barrier to the movement of large molecules between the blood and the tissue space. Of particular interest, under pathophysiological conditions, are the changes in vascular integrity that lead to the accumulation of fluid and macromolecules within the tissue space, resulting in changes in both tissue structure and function. The starting point for the investigation of these changes is an ability to characterize and assay tissue protein levels under controlled conditions. This has recently become possible with the introduction of large-pore dialysis membranes (11). Dialysate from naive skin contains between 0.25 and 0.3 mg/ml protein, ~70% of which is albumin. Although somewhat lower than that measured in peripheral lymph or in interstitial fluid using other, rather more traumatic techniques, it reflects the 20–30% dialysis efficiency of the 3,000-kDa membranes used in these studies and provides a reproducible measure of vascular integrity.

A century ago, William Bayliss showed that stimulation of primary afferent nerve fibers resulted in local vasodilatation. Subsequently, it was demonstrated in the skin that this was accompanied by an increase in local vascular permeability, leading to the accumulation of fluid, protein, and inflammatory cells within the tissue space (8). Key mediators of these responses have been shown to be neuropeptides, such as calcitonin gene-related peptide, a potent vasodilator, and substance P, which causes both an increase in vascular perfusion and in venular permeability (12, 14). Studies in animal models suggest that these neuropeptides cause vasodilatation by inducing the generation of secondary vasoactive mediators, including nitric oxide (4, 15). In humans, evidence for this remains controversial, with attenuation of neurogenic vasodilatation and protein extravasation by inhibitors of nitric oxide synthase and histamine H1 receptor antagonists being reported in some studies but not in others (Fig. 4). It is now possible to exploit the model of human skin to explore these mechanisms and indeed to begin to investigate the role of other novel peptides such somatostatin and the endomorphins in neurogenic inflammation.

**FIGURE 3.** Production of nitric oxide (NO) and cGMP in the weal-and-flare response to the intradermal injection of histamine in human skin. NO and cGMP were assayed in dialysate collected using a 2-kDa probe perfused at 5 μl/min with phosphate-buffered saline. Probes were inserted to run for 20 mm at a depth of ~0.6 mm below the epidermis (adapted from Ref. 4).

**FIGURE 4.** Effects of an H1 antihistamine on protein recovery in the dialysate from a histamine-induced weal in human skin in vivo. A 3,000-kDa probe was perfused at 3 μl/min with saline solution, to which was added histamine (100 μM) for a brief (30-s) period. Pretreatment with the H1 antihistamine cetirizine (100 nM) added to the probe perfusate significantly reduced the protein recovery (P < 0.01, Student’s t-test for paired data).

**Agonism and antagonism?**

Much of our knowledge about the mediator mechanisms underlying inflammatory and allergic responses has been gained from the use of pharmacological antagonists and inhibitors of the response in question. However, such approaches used in isolation provide only circumstantial evidence, which may not always be trustworthy. One example where the outcome of this approach has produced confusion rather than a correct answer has been the study of the effects of the so-called histamine H1 receptor antagonists. When it was initially observed that these drugs inhibited the vascular responses to other endogenous inflammatory mediators, e.g., bradykinin, as effectively as they do those to histamine itself (5), it was suggested that histamine was being released and acting as a final local mediator. However, microdialysis showed that this was not the case. Thus a more likely explanation is a complex interaction between histamine and bradykinin receptors at the endothelial cell membrane.

As a consequence of recent work, mainly using cloned receptors in reporter cells, there have been considerable advances in our knowledge of receptor function and the mechanisms by which receptor antagonists such as the H1 antihistamines have their effect. The outcome of these studies has been a questioning of the classic models of how G protein-coupled receptors work, including how they exert their effects at the vascular wall (9). Of particular relevance is the proposal that many of these receptors do not act as single entities but form dimers or even oligomers. Indeed, the postulate of a heterodimer formed between histamine H1 and bradykinin B2 receptors would explain the result described above.

Although still limited in the pharmacological and molecular biological tools suitable for use in humans, we can begin to address these novel proposals in a relevant human model by using the techniques described in this brief review. By comparison of the functional outcomes in vivo with information...
obtained in vitro in cell models, we can begin to explore the molecular mechanisms by which specific drugs have their anti-inflammatory effects, both at the vascular wall and beyond.

It must not be forgotten that cutaneous microdialysis has also been used, with some success, to explore the functional consequences of systemic or local exposure to xenobiotics and to investigate the influence of local vascular status on their pharmacokinetics (1). Of particular value has been the ability to deliver drugs into the tissue by reverse microdialysis with the simultaneous monitoring of drug responses in the target tissue. Thus, for example, at a single site on the forearm of a human volunteer it is now possible to deliver an anti-inflammatory agent (e.g., the H1 antihistamine cetirizine) by microdialysis to an inflammatory reaction (e.g., to allergen also delivered via the dialysis probe) while at the same time collecting the dialysate for assay of inflammatory cell products, secondary mediators, and extravascular protein levels as evidence of increased vascular permeability and monitoring the outcome of the reaction in terms of the changes in local blood flux and cutaneous sensation.

**Looking beyond the mirror**

People vary considerably in the inflammatory responses they mount against a noxious stimuli. Because of their ease of access and the opportunity they afford for accurate quantification, the in vivo models of inflammation in human skin described in this brief review are far superior to many of those used in other organ systems. We anticipate that, in the future, it will be possible to dissect out causes of interindividual variability in inflammatory responses by using these skin models. They may also be used to identify suitable therapeutic anti-inflammatory strategies and eventually to assess the concept of “individual patient”-oriented therapy.

We have not been able to reference the many and varied original studies in which skin vasoactivity has been explored using the techniques described in this review. We hope those authors will forgive us.

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**References**