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Original Publication:

Erik Tesselaar and Folke Sjöberg, Transdermal iontophoresis as an in-vivo technique for studying microvascular physiology, 2011, MICROVASCULAR RESEARCH, (81), 1, 88-96.

<http://dx.doi.org/10.1016/j.mvr.2010.11.002>

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Postprint available at: Linköping University Electronic Press

<http://urn.kb.se/resolve?urn=urn:nbn:se:liu:diva-66283>

Transdermal iontophoresis as an in-vivo technique for studying microvascular physiology

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Keywords: iontophoresis, laser Doppler, vasodilatation, vasoconstriction, endothelial function, vascular model

Abstract

Assessment of microvascular function is of major importance in understanding the physiology of the vasculature and in investigating the vascular effects of pathological conditions. Transdermal iontophoresis can be used to non-invasively introduce vasoactive drugs into the skin. The response of the local cutaneous microvasculature to these drugs can be measured by methods such as laser Doppler flowmetry. Although the technique has been used for over two decades, there are still important methodological issues to be resolved. This review describes the technique of iontophoresis as well as its development during recent years, while focusing on how iontophoresis can be used as an in-vivo model for studying physiologic mechanisms and on the analysis and interpretation of dose response data.

Introduction

It is important to understand the various mechanisms underlying the regulation of blood flow in the microvasculature in both health and disease, as tissue ischaemia is responsible for many pathological conditions. Experimental models therefore are not only important for the assessment of the microvascular physiology but also in studying the effects of drugs or drug candidates.

Studies of unprovoked vessels in its resting state generally do not contribute with much useful information about physiological or pharmacological mechanisms. Also, such measurements usually yield results with large variabilities due to temporal and spatial variations in vessel tension or blood flow (Tenland et al., 1983). Therefore, microvascular function is often studied in response to a stimulus – most commonly some sort of pharmacological or physiological provocation.

In vitro vascular models

The method that probably had the most impact on our current understanding of the basic vascular pharmacology and physiological functions of the microvasculature is the in vitro preparation of isolated vessels (Angus and Wright, 2000; Struijker-Boudier et al., 2007). The vessels, which are either harvested from animals or of human origin, are typically cut into segments of a few millimeters long and are mounted on hooks or sutured to two concentric pipettes and are directly coupled to a force transducer allowing wall force to be measured. Initially, the vessel segment is stretched to a certain level to generate a passive force equivalent to the conditions in vivo. Then, it can be activated by various external stimuli, such as vasoactive substances or pressure, while any change in tension or diameter is measured. This setup allows for precise studies of the reactions to these stimuli without mechanical and neural influences or the influence of circulating hormones and metabolites. The strength of this methodology in biology is clearly demonstrated by achievements such as the clarification of regulatory functions of the vascular endothelium (Furchgott and Zawadzki, 1980) and the discovery of the pathways of nitric oxide (Palmer et al., 1988).

In vitro experiments are complicated by the fact that they take place outside the body albeit in a controlled but otherwise artificial environment which makes it difficult to apply the results in a straightforward way to clinical situations. For example, before studying the response to a

vasodilator, the vessels have to be stretched to a certain preload level to simulate in vivo conditions, as they do generally not develop spontaneous tone (Schubert, 2005). This in itself confounds the responses of the vessels to the vasodilator as these responses are often dependent on the initial stretch of the vessel.

Increasing efforts are being directed towards the development of vascular models in which the vessels and vascular effects may be investigated in a tissue environment that incorporates the influences not only of the vessels themselves – as in the case of in vitro models – but also of nerve endings and mechanical and humoral factors (Angus and Wright, 2000). As such, these models provide a more integrative assessment of microvascular function as they elicit vascular responses that involve multiple-signaling pathways. Recent studies have indicated that changes in the cutaneous microvasculature often mirror changes in vessels of different size (Debbabi et al., 2010) and in other vascular beds, such as the coronary microcirculation (Khan et al., 2008). In fact, increasing evidence indicates that the cutaneous microcirculation can serve as a representative model to examine mechanisms of vascular function and dysfunction that occur on a systemic level (Holowatz et al., 2008; IJzerman et al., 2003).

Transdermal iontophoresis is a technique for controlled delivery of drugs into the skin, which has gained substantial interest over the past two decades as a method for studying microvascular function in vivo. Changes in the cutaneous microcirculation upon delivery of vasoactive agonists by iontophoresis with or without their respective antagonists, have been proven to give a lot of information about the underlying vascular physiology. The vasoactive drug most commonly delivered by iontophoresis is acetylcholine, often complemented by nitroprusside as a control drug. As acetylcholine causes vasodilation mainly through release of nitric oxide from the endothelium, it has been used to investigate endothelial dysfunction a large number of clinical conditions, the most important of which are diabetes and hypertension. Other vasoactive substances that have been given iontophoretically include noradrenaline, vasopressin and histamine.

This review will focus on how transdermal iontophoresis, combined with methods for skin blood flow measurement, can serve as a noninvasive in vivo equivalent of the classical vascular in vitro models, in which agonist/antagonist action is studied by measuring dose-dependent changes in vascular tension or diameter. First, the principles of iontophoresis will be shortly introduced as well as some of the current methodological issues. Then, common techniques to measure perfusion responses will be described. Finally, we will elaborate on

dose response modeling and the use of agonists and antagonists in iontophoresis studies, as we think that these aspects may make the technique more useful and powerful as an *in vivo* method for assessment of microvascular function.

Iontophoretic drug delivery

Mechanisms

A number of excellent reviews on iontophoresis as a method for enhanced transdermal drug delivery have been published earlier (Curdy et al., 2001; Dixit et al., 2007; Guy et al., 2000; Kanikkannan, 2002). Here, we will focus on the mechanisms and methodological issues relevant to the use of the iontophoresis in microvascular research.

During iontophoresis, an electric field is applied across the skin by means of two electrodes. The typical setup is schematically depicted in Figure 1. A drug, usually consisting of charged molecules, is placed under one or both electrodes such that electrorepulsive forces drive the drug molecules away from the electrodes, into the skin. This implies that a positively charged drug (dissolved in a vehicle solution) is placed under the positive electrode or anode, whereas a negatively charged drug is placed under the negative electrode or cathode. In this way, the rate of penetration of charged drugs through the relatively impermeable stratum corneum is greatly enhanced compared to passive diffusion.

Figure 1. Schematic representation of transdermal iontophoresis. D^+ and D^- are positively and negatively charged drug ions, respectively. V^+ and V^- are positively and negatively charged co-ions in the drug solution. A^+ and A^- are counter-ions traveling from the skin towards the electrode to maintain electroneutrality.

In practice (Figure 2), the drug ions are usually driven into the skin by a constant direct current although alternating currents or more complex current waveforms have also been used (Meyer and Oddsson, 2003; Reinauer et al., 1993). Current densities of up to 0.5 mA/cm^2 are considered safe (Ledger, 1992) but in practice current densities vary between $0.005 - 0.2 \text{ mA/cm}^2$.

Figure 2. Transdermal iontophoresis in practice. A current controller delivers a constant current to the skin by means of an active electrode, containing the substance to be introduced into the skin. This electrode in the image has an integrated laser Doppler probe. A passive electrode on the wrist is used to complete the electrical circuit.

The use of iontophoresis is not limited to charged drugs. At physiological pH, the skin acts as a negatively charged membrane. When an electric field is applied across such a membrane,

there is a net transport of water molecules across the skin, from the anode to the cathode. This electroosmotic flow results in an enhanced transport of dissolved uncharged molecules, especially if they are polar. Even though iontophoresis of uncharged molecules has been successfully applied both in diagnostic and therapeutic applications (Pikal, 2001; Potts et al., 2002), we know of no studies that have used electroosmosis as a transport mechanism for the delivery of vasoactive drugs for the assessment of microvascular function. The reason for this (Reinauer et al., 1993) may be that the transport of molecules by electroosmosis is relatively small compared to electrorepulsion, possibly precluding adequate vascular responses.

Transport pathways

How vasoactive substances delivered by iontophoresis find their way to the receptors responsible for the vascular responses, has not been studied specifically. However, considerable effort has been put into investigating through which pathways other, mostly therapeutical drugs are transported into the skin during iontophoresis. It is today recognized that the pathways depend on the properties of the drug molecules. For charged drugs, which are commonly used in microvascular iontophoresis studies, the predominant route of transport seems to be through hair follicles and sweat ducts, whereas neutral, lipophilic drugs are primarily transported through intercellular spaces of the stratum corneum (Cullander, 1992; Singh and Maibach, 1996; Turner and Guy, 1997). Also, the use of pilocarpine in the diagnosis of cystic fibrosis suggests in itself that drugs travel through sweat ducts. In Figure 3, the transport pathways involved during iontophoresis are visualized using confocal fluorescence imaging.

While the heterogeneity seen in the microvascular responses is usually explained by the differences in capillary density throughout the skin (Braverman, 2000; Wardell et al., 1994), it may also be caused by the fact that drug molecules are transported through appendages such as the hair follicle. The base of the hair follicle is highly vascularized and contains many nerve endings. It is therefore likely that any vascular responses seen during iontophoresis originate at these distinct locations, followed by a further spreading of the response as drugs find their way through the cutaneous microvasculature (Figure 6).

Delivered drug dose

One of the important limitations of iontophoresis as a drug delivery method is the fact that the delivered drug dose is unknown and can only be estimated. By applying a constant electric

current for a certain time, a iontophoretic charge (Coulombs) is delivered, which is defined as current strength (ampères) $\times$ duration (seconds). This iontophoretic charge is generally used as an estimate for the dose administered to the local microvasculature. The rationale for this assumption is that a constant part of the electric current is carried by drug ions while another part is carried by other ions, such as background ions in the drug solution or counter ions (primarily small ions such as Na^+ , K^+ , Ca^+ and Cl^-) that travel from the skin towards the electrode. This is the case for most inorganic ions and drug ions in in vitro iontophoresis models (Phipps et al., 1989).

Figure 3. Confocal fluorescence images obtained following iontophoresis of calcein for 4 hours, showing that the charged fluorophore is predominantly transported through hair follicles. Images were obtained at a depth of 10 μm below the skin surface (left); at 20 μm below the skin surface (middle); and at 30 μm below the skin surface (right). Redrawn from (Turner and Guy, 1997)

The situation during transdermal iontophoresis is however essentially different from in vitro circumstances. First, the transport number of the drug ions (the relative amount of current carried by them) is usually unknown, and is dependent on the drug formulation and other factors. This largely prohibits estimation of absolute drug doses. Furthermore, even when the influx of drugs is considered a zero-order infusion process, there is a simultaneous removal of drug ions out of the microvascular bed as a result of diffusion and active transport by local blood flow (Figure 4). This implies that the drug dose in the microvascular bed is not linearly proportional to the iontophoretic charge in a typical protocol for iontophoresis of vasoactive drugs. Also, it is likely that the true physiological maximum response to the drug is never reached during the experiments because the maximum concentration of the drug in the skin is limited by the local removal of the drug. This is more likely to be a problem with vasodilator

drugs than vasoconstrictors, since the removal by local blood flow is increased due to the action of the vasodilator itself (Tesselaar et al., 2009).

Figure 4. Schematic representation of drug kinetics during iontophoresis, incorporating the drug transport into and out of the microvascular bed and the effect on local blood flow. As a result of these drug kinetics, the relation between iontophoretic charge and drug dose may be complicated. Despite this, the E_{max} model has been applied to blood flow dose responses during iontophoretic drug delivery (Tesselaar et al., 2009).

Drug delivery protocols and data analysis

Protocols for drug delivery can be divided in continuous current protocols and protocols involving multiple current pulses separated by current-free intervals. Early studies applying iontophoresis for microvascular assessment used multiple pulses, but over time, both approaches have become common.

The analysis of laser Doppler blood flow data during and after iontophoresis has been done in various ways (Figure 5). Some investigators have used the absolute increase in perfusion, with or without subtraction of baseline perfusion values. Others have expressed the responses as change with respect to baseline. Besides maximum responses (Abou-Elenin et al., 2002; Newton et al., 2001), the parameter of interest has been the area under the response curve (AUC) after a certain period of iontophoretic current application (Anderson et al., 2004; Asberg et al., 1999). However, both approaches have limitations. First, the maximum response seen during an experiment may not reflect the true physiological maximum. Second, the response over time to a drug that is applied by iontophoresis contains more information than the maximum response alone and by considering only the maximum response, important physiological characteristics may be overlooked. Therefore, many investigators have used multiple pulses and measured the perfusion response to increasing iontophoretic charges (Asberg et al., 2000; Christen et al., 2004; Khan et al., 2000; Morris et al., 1995).

When perfusion responses are measured after multiple iontophoretic charges, comparison of responses between drugs or between patient groups have most often been analyzed using multiple-way analyses of variance (ANOVA). A limitation of this approach is that ANOVA ignores the fact that doses or time points come in order and that it does not recognize trends. ANOVA may therefore not really address the question underlying the experiments.

When drugs are delivered by a single, continuous current pulse and blood flow measurements are made at regular intervals during current application, the obtained responses can be analyzed as time response curves or, when proportionality between time and dose is assumed, as dose response curves. Strictly though, these dose response curves cannot be compared to conventional dose responses as obtained *in vitro*. The reason for this is twofold. First, the response to the drug at any moment during current application is probably not fully maximized due to the limited rate of transport through the skin. Therefore, dose response curves obtained with continuous iontophoresis will likely be right-shifted compared to absolute dose response curves. Also, the drug dynamics in the target area and the effect on local drug dose, as described above, are likely to influence the shape of the dose response curves.

When the aim is to mimic the technique of *in-vitro* vessel preparations, the application of multiple current pulses seems the preferred method. Such protocols make it possible to measure the maximum response after each iontophoretic charge and therefore, cumulative

dose response curves can be made, in a rather similar way as is done in-vitro. However, a practical drawback of multiple-pulse protocols is that a substantial time is needed for the response to reach its peak with slow-acting drugs, which can make protocols lengthy. Importantly, even with multiple pulse protocols, it can be questioned to what extent the protocols result in “true” cumulative dose response curves, as we know them from in vitro methodology, due to the aforementioned complicated relation between time and dose in iontophoresis in vivo. In either case, expressing the blood flow data as time responses enables a more straightforward analysis of the responses to the delivered drugs.

Figure 5. Different methods to analyze and compare blood flow responses obtained during iontophoresis of vasoactive drugs. Comparison of maximum responses (A), analysis of multiple responses at increasing iontophoretic charges (B), time response curves (C) and dose response analysis, with Emax model fitted to the data (D). Graphs are based on typical data, in which the treatment gives a 20% reduction in maximum response.

Even though dose response curves obtained by iontophoresis are likely to be different from the conventional dose responses known from in vitro methodology, recent studies have shown that blood flow responses can well be characterized by a general pharmacodynamic dose-response model, at least for the most commonly used vasodilators: acetylcholine and sodium nitroprusside (Henricson et al., 2007). A major strength of dose response analysis is that it allows for interpretation of responses in terms of conventional pharmacodynamic parameters such as ED₅₀ and maximum response. Also, it enables a quantitative, statistically supported comparison of well-defined parameters, for example between patient groups or between different drugs (Gabrielsson and Weiner, 1999).

Nonspecific vasodilatation

Another important property of transdermal iontophoresis is that it may elicit a so-called 'nonspecific response' (galvanic response, current – induced response, method – induced response). This nonspecific response, which is seen during iontophoresis of non-vasoactive compounds such as deionized water, tap water, and sodium chloride, has received considerable attention since it was first investigated by Grossmann et al. (Grossmann et al., 1995). They found an increase in blood flow upon iontophoresis of sodium, lithium, chloride, nitride, acetate and bicarbonate ions. This increase in blood flow was attributed to membrane hyperpolarization and pH changes induced by the ions in the vehicle. The nonspecific response at the cathode was found stronger than the response at the anode. Since then, a number of studies have investigated the nonspecific effect, and several explanations have been given (Table 1), including the involvement of prostaglandins (Berliner, 1997; Durand et al., 2004), and sensory nerve stimulation (Morris and Shore, 1996), particularly the axon reflex in primary afferent fibers (Tartas et al., 2005).

The mechanisms responsible for nonspecific vasodilatation during iontophoresis are not completely understood. However, nonspecific vasodilatation is an important confounding factor when iontophoresis is used to study the microvasculature, because it introduces a blood flow effect that is not a result of the drug under study. Various strategies have therefore been applied to avoid nonspecific effects, including the use of local anesthetics (Morris and Shore, 1996), special vehicle solutions (Noon et al., 1998) and the limitation of current strength or total iontophoretic charge (Droog et al., 2004).

Measurement of vascular responses

Vasodilatation

The microvascular responses to the drugs applied using iontophoresis have most often been measured using laser Doppler flowmetry (Bonner and Nossal, 1981; Humeau et al., 2007; Leahy et al., 1999). Laser Doppler flowmetry and iontophoresis were first combined by Ekenvall and Lindblad (Ekenvall and Lindblad, 1986) who administered the vasoconstrictor norepinephrine. The technique is based on the measurement of the shift in frequency of laser light that has been scattered by moving red blood cells (RBCs) in the tissue. The signal that is obtained is proportional to the perfusion (defined as RBC concentration multiplied by RBC velocity), and is either expressed in volts or arbitrary units.

Several types of laser Doppler equipment can be distinguished. The two types that have been used the most are single-site laser Doppler flowmetry (LDF) and laser Doppler perfusion imaging (LDPI). When blood flow during or after iontophoresis is assessed by LDF, a probe containing the laser and the detector is integrated with the iontophoresis chamber and blood flow is measured continuously at a single spot of about 2-3 mm in diameter. The probe is in direct contact with the skin while the drug solution is applied on the skin area surrounding the probe. When LDPI is used, a scanner head containing a laser source is placed at a distance of typically 15 cm above the site of iontophoresis. The laser scans the iontophoresis area during and after drug delivery, as it penetrates the drug solution as well as a thin glass slide which keeps the drug solution in the chamber and prevents scattering artifacts from the fluid surface. This results in an array of perfusion values, which is often averaged to yield a single, mean, perfusion value for each scan.

The advantage of LDF is the high temporal resolution of the perfusion measurement. Blood flow response can be assessed in real time while the drugs enter the skin during iontophoresis. However, the microvasculature in the skin is highly heterogeneous (Wardell et al., 1994) and so are the perfusion responses to iontophoresis of vasoactive drugs. The responses tend to start at distinct skin sites, and then spread out slowly across the adjacent tissue (Droog and Sjöberg, 2003). This effect is possibly caused by the fact that the transport of drugs takes place through distinct appendages in the skin (Figure 6).

Therefore, an advantage of LDPI compared with LDF is that the blood flow response is measured over the whole area where the drug is delivered, enabling an averaged measure as

well as information about the heterogeneity. On the other hand, scanning the area of iontophoresis typically takes a few seconds, depending on the spatial resolution and the properties of the scanning system. Therefore, the temporal resolution is reduced. As a result, temporal variations in perfusion may end up as spatial heterogeneity in the perfusion images, although this usually is not a problem considering the small areas of iontophoresis electrode chambers.

Figure 6. Typical sequence of laser Doppler perfusion images obtained during iontophoresis of sodium chloride, showing a highly heterogeneous blood flow response within the electrode chamber.

Recently, another laser Doppler-based technique has become commercially available. The technique in theory combines the advantages of single-point LDF and scanning LDPI techniques, as it provides instantaneous recording of the perfusion in an area of tissue. Laser Speckle Contrast Imaging is based on the analysis of speckle contrast and, like LDF and LDPI, provides a perfusion measure that is proportional to the concentration and the average velocity of red blood cells (Boas and Dunn, 2010; Briers, 2001; O'Doherty et al., 2009).

Vasoconstriction

Most investigations made using iontophoresis and laser Doppler flowmetry have been performed with vasodilators and only a few include the effects of vasoconstrictors. This overrepresentation is most likely due to the low sensitivity of the laser Doppler technique in measuring baseline or decreased skin perfusion (Lipnicki and Drummond, 2001). This low sensitivity is caused by at least two effects. In its resting state, the cutaneous microcirculation has a relatively low perfusion, with perfusion values that are typically close to the biological zero (the contribution of the flow signal caused by the natural movement of molecules red blood cells in the tissue (Kernick et al., 1999). Second, when there is vasoconstriction in the capillary bed, the laser light, instead of being absorbed by RBC's in the capillaries, penetrates to deeper areas such as the subdermal plexus, where the changes in flow may be small (Fredriksson et al., 2009). These effects make assessment of vasoconstrictor responses difficult to perform using laser Doppler based perfusion detectors.

Figure 7. Number of articles published in the past 25 years in which iontophoresis is used for assessment of microvascular function, specified for the most commonly used drugs.

Figure 8. Perfusion response to acetylcholine during 10 minutes of air breathing and 100% oxygen breathing without pretreatment (A) or after pretreatment with aspirin (B). The blunted response to acetylcholine seen during breathing of 100% oxygen is restored under aspirin pretreatment, indicating that the effect of hyperoxia on endothelium-dependent vasodilatation involves prostaglandins. Taken from (Rousseau et al., 2010).

An alternative to the laser Doppler technique has been introduced recently, which is based on polarization spectroscopy imaging and which is particularly suitable for measuring vasoconstriction responses. In contrast to LDF, it measures RBC concentration in the skin (O'Doherty et al., 2007). Initial investigations show that the technique offers improved

sensitivity in measuring vasoconstriction in the skin during iontophoresis, although more research is needed to validate the performance of the technique against the traditional laser Doppler-based methods of perfusion measurement (Farnebo et al., 2010; Petersen et al., 2010; Zhai et al., 2009).

Studying vascular physiology

The number of studies in which iontophoresis is used to investigate microvascular function has increased continuously during the past 25 years. Currently, a PubMed search on the terms “iontophoresis” and “laser Doppler” yields over 300 publications. In the vast majority of studies, acetylcholine and nitroprusside are delivered to assess endothelial function. Iontophoresis of these two drugs has become an established method to assess endothelial function. Besides acetylcholine and nitroprusside, catecholamines are quite often used to test responses during thermal stress or in studies on neurovascular disorders. A limited number of studies has been performed with other substances, such as histamine, insulin, vasopressin, endothelin, pilocarpine and adrenaline.

Along with these agonists, various antagonists and physiological provocations, have been applied in parallel to iontophoresis to investigate specific mechanisms. Several approaches are possible to study these mechanisms. Both the agonist and antagonist can be delivered through the iontophoresis electrode (simultaneously or sequentially). If the antagonist is not suitable for iontophoretic delivery, the oral or intravenous routes can be chosen. Recently, a number of studies have combined iontophoresis with the technique of microdialysis, where the microdialysis catheter is placed intradermally under the iontophoresis electrode to deliver substances intended to interact with the iontophoretically applied drug (Hodges et al., 2006; Yamazaki, 2007).

Endothelial function

Acetylcholine (ACh) is a muscarinic receptor agonist that has been comprehensively studied in isolated vessel preparations. In 1980, Furchgott and Zawadzki demonstrated that the relaxation of vascular smooth muscle cells in response to ACh is dependent on the anatomical integrity of the endothelium (Furchgott and Zawadzki, 1980). Later, nitric oxide (NO) was identified as the substance that is released from the endothelium, producing smooth muscle relaxation (Ignarro et al., 1987). NO is produced enzymatically from the amino acid L-arginine by enzymes termed eNO synthases (NOS). In the cutaneous microvasculature,

vasodilatation in response to acetylcholine may be mediated by mechanisms other than NO, although the results are conflicting. Prostaglandins may (Khan et al., 1997; Lenasi and Strucl, 2008; Noon et al., 1998) or may not be involved (Berghoff et al., 2002; Morris and Shore, 1996). Also, it has been suggested that acetylcholine induces vasodilatation through stimulation of sensory nerves (Berghoff et al., 2002). Despite conflicting evidence regarding the mechanisms by which the drug causes vasodilatation, ACh is commonly delivered iontophoretically to test endothelial function in a variety of clinical conditions, including diabetes (Arora et al., 1998; Cohen et al., 2008; Hannemann et al., 2002; Morris et al., 1995), hypertension (Cupisti et al., 2000b; Farkas et al., 2004; Kellogg et al., 1998; Monostori et al., 2010), pre-eclampsia (Blaauw et al., 2005; Eneroth-Grimfors et al., 1993; Khan et al., 2005), Raynauds phenomenon (Anderson et al., 1996; Hettema et al., 2009; Khan and Belch, 1999), renal failure (Cupisti et al., 2000a), HIV (Monsuez et al., 2000) and heart failure (Andersson et al., 2005; Balmain et al., 2007). Also, it has been applied to investigate the effects of smoking (Pellaton et al., 2002), age (Rossi et al., 2002a), gender (Algotsson et al., 1995), hyperoxia (Rousseau et al., 2010; Yamazaki, 2007) and exercise (Lenasi and Strucl, 2004; Rossi et al., 2002b).

Various antagonists have been used to study their effect on the vascular response to acetylcholine. An example is aspirin (acetylsalicylic acid), a noncompetitive, irreversible antagonist of the enzyme cyclooxygenase that inhibits the synthesis of prostaglandins. Oral pretreatment with aspirin has been used often to investigate the involvement of prostaglandins in the response to various vasodilatory stimuli. For instance, prostaglandins have been found to play a role in the endothelium-dependent, vasodilatory response to acetylcholine but also in the nonspecific response to iontophoretic current. Furthermore, the blunted response to acetylcholine seen during breathing of 100% oxygen is restored under aspirin pretreatment, indicating that the effect of hyperoxia on the endothelium-dependent vasodilatation involves prostaglandins (Rousseau et al., 2010). Similarly, the use of aspirin as an antagonist during iontophoresis of acetylcholine has suggested the involvement of prostanoids in altered endothelial function in women with Raynauds disease (Easter and Marshall, 2005).

Another antagonist that has been applied is atropine (Figure 9), which blocks muscarinic receptors resulting in a rightward shift and suppression of the vasodilatory dose response to acetylcholine, when both substances are delivered using iontophoresis (Grossmann et al., 1995; Henricson et al., 2007).

Figure 9. Blood flow response to iontophoretically applied acetylcholine (55 mmol/l) after pretreatment with the antagonist atropine ($n = 5$). Dashed line represents pretreatment with 1.14 mmol/l atropine, dotted line represents pretreatment with 0.114 mmol/l atropine. Solid line represents the blood flow response without pretreatment. Taken from (Henricson et al., 2007).

When studying endothelial function, it is often desirable to administer exogenous NO as a control measure. Sodium nitroprusside (SNP), a drug that is used clinically to reduce blood pressure, e.g. in hypertensive emergencies, is used for this purpose. NO is released from the nitroprusside molecule when it reacts with sulphhydryl-containing compounds present in the tissue, such as cysteine (CySH) and glutathione (GSH). In presence of GSH, a chemical reaction leads to formation of GSH^+ , which in turn leads to the formation of GSNO and finally to the release of NO (Grossi and D'Angelo, 2005). Since the vascular response elicited by SNP is independent of endothelial function, it is considered a measure of general vasodilatory capacity.

Skin thermoregulatory control

The thermoregulatory control of human skin blood flow is important to the maintenance of normal body temperatures (Charkoudian, 2003) and is controlled by sympathetic neural mechanisms including the noradrenergic vasoconstrictor system. This system can be studied by measuring the responses to iontophoretic delivery of selective alpha-1 and alpha-2 agonists with or without their respective antagonists. Such iontophoresis studies have been done to investigate local or whole body heating (Kellogg, 2006; Kellogg et al., 1991) and local cooling (Hodges et al., 2006; Lindblad and Ekenvall, 1990; Pergola et al., 1993).

An example of the use of iontophoresis in the investigation of thermoregulatory function is a study (Kellogg et al., 1989), in which bretylium tosylate was delivered to the skin. Bretylium, which blocks the release of noradrenaline from nerve endings, was found to abolish the cutaneous vasoconstriction induced by cold stress, while it did not alter the vasodilator response induced by heat stress. This confirmed previous indications that the cutaneous vasculature is controlled by a noradrenergic vasoconstrictor system and a nonadrenergic active vasodilator system.

Other drugs

Besides the studies on endothelial function and thermoregulatory function, a few other studies have been performed applying a variety of substances using iontophoresis. Histamine has been delivered by iontophoresis to study the effect of antihistamine drugs (Leroy et al., 1998), to analyze neurogenic flare reactions (Bickel et al., 2002) and to assess the pharmacodynamics of histamine itself (Jones et al., 2009). Iontophoresis has also been used to study the effect of exercise (Rossi et al., 2005) and obesity (de Jongh et al., 2008) on the local microvascular responses to insulin as well as to investigate the role of calcitonin gene-related peptide in migraine (Edvinsson and Edvinsson, 2008; Rossi et al., 2007).

Conclusion

The method of transdermal iontophoresis of vasoactive substances has, during the past 25 years, continuously been investigated and characterized. There is increasing evidence that changes in the cutaneous microvascular function are related to changes in general microvascular function in a number of clinical conditions. These facts have made transdermal iontophoresis of vasoactive substances an established noninvasive in vivo model for studying microvascular function.

Despite its success, there are limitations and methodological issues that have to be further investigated for the technique to become more valuable and accepted as a complementing technique to the highly established in vitro models. The fact that the drug dose in the tissue during iontophoresis is unknown, regardless of the delivery regime, is perhaps the major limitation of the method in comparison with in-vitro methodology. A better understanding of the local drug kinetics and response dynamics during iontophoresis of vasoactive drugs is needed to enable more accurate dose response analysis. This understanding could be gained by more sophisticated models of the transport of drugs and the dynamics of the responses.

Finally, the recent developments of more sensitive and reproducible techniques for measurement of microvascular blood flow during iontophoresis may lead to further improvements in the assessment of microvascular function in vivo.

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Table 1

Possible mechanism	Evidence	References
membrane hyperpolarization pH changes	difference between anodal and cathodal iontophoresis	(Grossmann et al., 1995) (Droog and Sjoberg, 2003) (Asberg et al., 1999) (Ferrell et al., 2002)
vasoactive substances in sweat ducts	drugs travel primarily through sweat ducts	(Morris and Shore, 1996)
sensory nerves	EMLA partially blocks the response	(Morris and Shore, 1996)
prostaglandins	aspirin partially blocks the response	(Berliner, 1997) (Durand et al., 2002)
small primary afferent fibers	capsaicin partially blocks the response	(Durand et al., 2002)

Table 1. Overview of the mechanisms that have been suggested to cause nonspecific vasodilatation during iontophoresis.