A Review of Cutaneous Microdialysis of Inflammatory Dermatoses

Helen REA and Brian KIRBY
St Vincent's University Hospital, Dublin, Ireland

Microdialysis is a minimally invasive technique to study metabolic, biochemical, and pharmacological events in tissue. Factors that influence microdialysate collection include molecular weight cutoff of the membrane, perfusion rate, perfusate viscosity, duration of collection, depth of the catheter, length of the tubing and adsorption of hydrophobic molecules to the membrane. To standardize these factors, a robust sampling protocol needs to be established. Microdialysis is applied in healthy and inflamed skin. It enables the *in vivo* sampling of endogenous and exogenous substances in skin's extracellular fluid. In atopic dermatitis, levels of neuropeptides, eicosanoids and histamine pre- and post-treatment treatment have been conducted. Microdialysis in atopic skin has assessed the pharmodynamics of a number of topical drugs. In psoriatic skin, the 'cytokine fingerprint' has been evaluated through microdialysis and bioassays. This unique fingerprint has also been analyzed after certain pharmacological treatments for psoriasis.

Key words: cutaneous microdialysis; inflammation; atopic dermatitis; psoriasis; cytokines.

Accepted May 22, 2019; E-published May 23, 2019

Acta Derm Venereol 2019; 99: 945–952.

Corr: Dr Helen Rea, St Vincent's University Hospital, Dublin 4, Ireland. E-mail: helen.rea@ucdconnect.ie

SIGNIFICANCE

This review summarises the role of microdialysis of the skin. It is a useful tool to evaluate the skin's biological processes in both healthy and inflamed skin. It can also enable a better understanding of the mechanism of action of certain drugs in inflamed skin. The microdialysis technique still needs to be standardised so that its practice can be used in a reproducible fashion in clinical studies. It would be interesting to carry out microdialysis in subjects with the skin condition, hidradenitis suppurativa as this research has not yet been conducted.

Microdialysis is a minimally invasive technique for the chronological study of metabolic, biochemical, and pharmacological events in living tissue (1). Microdialysis was introduced approximately 40 years ago as a method for monitoring neurotransmitters in the brain of rodents (2, 3). The first published application in humans was a study on interstitial glucose in 1987 (4). During the last two decades, microdialysis has become a well-established method for continuously sampling substances within the extracellular fluid compartment of various tissues outside the central nervous system, including subcutaneous adipose tissue, dermis, muscle and other organs (5, 6).

The usefulness of microdialysis lies in its measurement of change. This change can be provoked by spontaneous events (e.g. the metabolic deterioration in a patient in intensive care (7)) or by the topical or systemic administration of a provoking agent (e.g. oral ingestion of antimicrobial or anti-neoplastic agents and subsequent measurement of their concentrations.). It can also monitor the change evoked by administration of an active agent administered by the microdialysis catheter itself, through reverse microdialysis, which shows changes in the concentration pattern of metabolites and drugs *in situ* (8).

APPLICATIONS OF MICRODIALYSIS

Microdialysis has many useful applications. It plays a role in neuroscience and predominantly in animal studies, enabling the measurement of neurotransmitters, small neuromodulators, amino acids and energy metabolites (9). Exogenous drugs which can be analysed by microdialysis include anti-depressants and anti-psychotics and other drugs that act on the central nervous system. Glucose concentrations in patients with diabetes (5, 10) can take place through intravascular and subcutaneous microdialysis catheter placement. Cardiac troponin has been measured in the myocardium of cardiac surgery patients (11). In the skin, the bioavailability of both topical drugs (particularly non-steroidal anti inflammatory drugs (12, 13) and systemic drugs, as well as the measurement of metabolic changes can be assessed.

Despite extensive use of microdialysis in the studies of inflammation of the skin, comparative studies with histology, plasma levels and other models are sparse. Sjogren & Anderson compared cytokine findings in the skin by microdialysis and biopsies and found moderate to excellent agreement (14). Further methodology studies may be needed to improve the translational value of microdialysis. Groth et al. (15) deduced that microdialysis technique is probably primarily useful for the study of hydrophilic substances and substances with low molecular weight and low protein binding. Since this study, Clough (16) has conducted further studies on microdialysis of large molecules. She noted that the protein dialysate level of macromolecules was significantly higher in the first 5 min of collection compared to those

doi: 10.2340/00015555-3223
Acta Derm Venereol 2019; 99: 945–952

collected after this. This is related to a large concentration gradient between the perfusate and interstitial space, and a very fast diffusion rate as a result. In addition, the application of cutaneous microdialysis for the study of lipophilic substances needs further methodological development. Many lipophilic substances adhere to the polymeric microdialysis membrane and tubing, thus yielding lower relative recoveries of dialysate. However, Sun & Stenken (17) enhanced the microdialysis recovery of the lipophilic eicosanoids significantly through the addition of cyclodextrins or organic substances such as arachidonic acid to the perfusate.

THE MICRODIALYSIS TECHNIQUE IN SKIN

Microdialysis equipment includes a membrane, a catheter, a pump, a guide needle, a sampling device and a perfusion fluid (usually normal saline) with a physiological buffer. The microdialysis technique is minimally invasive and requires the insertion of a small microdialysis catheter into the tissue of interest. The microdialysis catheter consists of a semi-permeable membrane that is continuously perfused with the physiological solution. This physiological solution is called a 'perfusate', and then becomes a 'dialysate' once it has filtered through the membrane. Pumps that are capable of low (0.1–8 µl/min) (1) but consistent speeds are required. This allows for the passive diffusion of substances. The size of the substances diffused depends on the permeability of the catheter. Depending on the size that you wish to analyse, catheters are used that allow diffusion of substances with cut offs between between 3,000 to 120,000 Daltons (9). The direction of the analysate flow is determined by the respective concentration gradient and allows the usage of microdialysis probes as sampling (retrodialysis) as well as tools for the delivery of investigated molecules or medication. The solution leaving the probe (dialysate) is collected at certain time intervals for analysis. 'Retrodialysis' (18) occurs when diffusible compounds are added to the perfusate, and can diffuse from the catheter into the tissue of interest, exerting their effects.

The design of microdialysis probes is divided into two basic categories: linear and concentric. The linear style probe is a membrane embedded within a length of small diameter tubing. It is usually used for tissue implants such as those in the subcutis (19), dermis (20), adipose tissue or muscle (21). Concentric probes have a membrane located at the distal end of a supporting cannula. They are most often used for accessing the extracellular fluid of the brain (22, 23). This avoids unnecessary invasiveness at this site as this probe has only one insertion point, and no exit point.

The microdialysis probe tip is inserted along the skin approximately 1 cm from the point of insertion using a guide needle. The bevel should reach the depth of the dermo–epidermal junction. The membrane is then perfused with the physiological solution at a fixed perfusion rate, usually between 2 and 5 µl/min. Effects of the probe insertion can lead to direct trauma to cells, a foreign body inflammatory reaction, including the release of interleukins (ILs) and tumour necrosis factor (TNF)-α, and circulatory effects over a wider area by axon reflex. Anderson et al. (24) used laser Doppler perfusion imaging to investigate skin blood perfusion with skin microdialysis. They concluded that increases in skin blood perfusion tend to subside around 15 min after probe insertion and by 60 min values are near resting levels. Thus, collection of the dialysate must take place at least 15 min after probe placement, however optimal collection is after 60 min (**Fig. 1**).

Sample analysis is a critical step in microdialysis and various methods exist. These include enzyme-linked immunosorbent assays, radioimmunoassays and high performance liquid chromatography. Other approaches include microsphere-based protein micro-assays, electron spin resonance, immunoaffinity capillary electrophoresis, capillary electrochromatography or mass spectrometry-based proteomics (25, 26).

FACTORS INFLUENCING DIALYSATE COLLECTION

Certain environmental factors can influence dialysate collection so these must be managed to ensure steady state elution. The relative recovery for different molecules can differ according to their temperature, which is directly proportional to their diffusion coefficient (27). *In vivo* microdialysis should be carried out at a constant body temperature of 37°C. Blood flow also influences the steady state concentration (28). Clough et al. (28) found that the magnitude of relative recovery *in vivo* is directly related to blood flow. This was exemplified when the relative recovery of the small diffusible molecule, sodium fluorescein was reduced by 50% through the application of a local vasodilator, glyceryl trinitrate patch. The pH values (29) of the medium surrounding

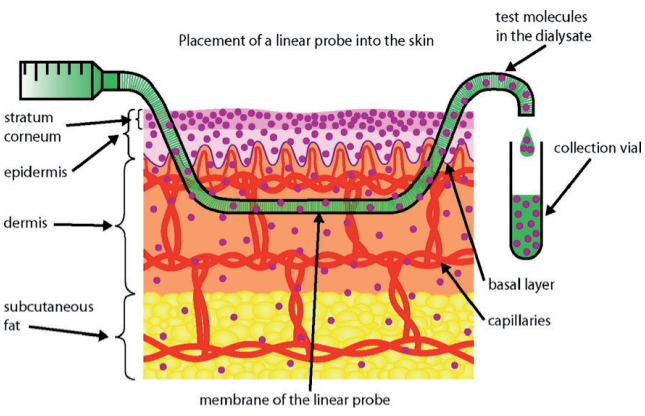

Fig. 1. The apparatus arrangement of a microdialysis membrane in the skin. From Ref. 13 after permission from the publisher.

the catheter influences the relative recovery of certain cytokines. *In vitro*, the RR of IL-10 and TNF-α varied significantly for pH 5 and 8 respectively, compared to pH 7 ($p<0.05$). However, *in vivo* the pH of interstitial fluid is normally 7 and this is not normally a variable.

Mechanical factors that affect collection and analysis include the molecular weight cut off of the membrane, the perfusion rate (30) (as previously discussed), perfusate fluid viscosity, the duration of collection, the depth of the catheter probe in the skin, the length of the tubing and adsorption of hydrophobic molecules to the polymeric materials in the probe and on the membrane.

The higher the molecular weight membrane cut-off of the microdialysis probe, the higher the fluid loss. Unfortunately, many of the mediators of pathological conditions of the skin are higher molecular weight peptides and proteins (5). These membranes lead to greater bulk fluid flow either in to the probe (ultrafiltration) or out of the probe (probe buffer loss.) It has been documented that significant probe fluid loss (up to 50% of expected volume) is a consequence of microdialysis in protein-containing solutions (31).

Lowering the molecular weight cut-off of the membrane can yield more dialysate volume but this limits the collection of metabolically active substances. There are two possible alternatives to overcome this problem. The first is to alter the fluid viscosity in the perfusate by adding a protein such as bovine serum albumin (BSA) to minimise the fluid loss from the microdialysis probe (32). The size of BSA prevents its diffusion across the semipermeable membrane of the microdialysis probe by placing an osmotic pressure that can offset the hydrostatic pressure forcing fluid out of the probe. The second possible solution to this problem would introduce the use a 'push-pull' pumping system (33). The push-pull pumping system is set up so that the ingoing pressure to the catheter is directly balanced against the outgoing pressure.

Poiseuille's law applies to microdialysis collection. This states that the velocity of the steady flow of a fluid through a narrow tube (such as a catheter) varies directly as the pressure (hydrostatic pressure in the extracellular fluid space) and the fourth power of the radius of the tube and inversely as the length of the tube and the coefficient of fluid viscosity (**Fig. 2**).

Q	Flow rate
P	Pressure
r	Radius
η	Fluid viscosity
l	Length of tubing

$$Q = \frac{\pi \mathrm{Pr}^4}{8\eta l}$$

Fig. 2. Poiseuille's aw applies to Cutaneous Microdialysate Collection. This states that the velocity of the steady flow of a fluid through a narrow tube (such as a catheter) varies directly as the pressure (hydrostatic pressure in the extracellular fluid space) and the fourth power of the radius of the tube and inversely as the length of the tube and the coefficient of fluid viscosity.

To standardize these factors, a robust sampling technique is still to be established. A slower perfusion rate equates to less fluid loss so standardization is recommended at rate of 3–5 µl/min. The depth of the catheter probe in the skin can be standardised using a Doppler ultrasound (Dermascan A Sonotron; AB Sweden) (24). Of note, there was no statistical difference found between continuous and intermittent versus continuous only microdialysis (34) thus continuous microdialysis is the standard of practice.

PROBE CALIBRATION METHODS

The following techniques are employed to calculate the tissue uptake or delivery of substances. When the probe is used in sampling mode, the microdialysate concentration is usually a fraction of the unbound concentration in the tissue. This is known as the relative recovery. To calculate the relative recovery from the tissue in this mode, the following equation is used;

RR gain= C dialysate/C tissue, (Equation 1)

where RR (gain) is the relative recovery of the tissue uptake, C dialysate is the concentration of the dialysate and C tissue is the concentration of the tissue of interest.

Of note, an *in vivo* study of long term microdialysis comparing the relative recovery in linear versus concentric catheters, there was a statistically significantly lower RR in the case of linear catheters (73.3% versus 84.9%) (35).

A number of microdialysis calibration methods can be used depending on the goal of the experiment. Calibration methods in the literature are retrodialysis (36), ultraslow flow rate (37), no net flux (4) and dynamic no net flux methods (37). A good calibrator should have similar diffusion, transport and metabolism properties to the solute of interest.

The most common calibration mode is retrodialysis, which is used for drug delivery. In order to calculate the drug in the probe diffusing in to the surrounding tissue, the following equation is used;

RR loss=(C perfusate – C dialysate/C perfusate), (Equation 2)

where, RR (loss) is relative recovery (lost from the tissue), C perfusate is the concentration of the perfusate and C dialysate is the concentration of the dialysate.

APPLICATIONS OF MICRODIALYSIS IN HUMAN SKIN

Microdialysis can be used in healthy skin to monitor endogenous metabolites and their response to homeostatic

perturbations such as ultraviolet radiation light, cold or pain. In addition, it is being increasingly employed to monitor exogenous substances such as free, unbound drug tissue concentrations.

Microdialysis has been extensively used in healthy skin and allows for the *in vivo* sampling of both endogenous and exogenous substances in the extracellular fluid, which represents up to 20% of the tissue volume. The endogenous substances include neuropeptides (such as substance P and calcitonin gene-related peptide (38, 39), insulin (5), cytokines (40), TNF-α (41), leukotrienes (20), eicosanoids (42), nitric oxide (43), histamine (44) and growth factors.

Falcone et al. (45) recently explored 10 minimally invasive methods of sampling IL-1α and interleukin-1 receptor antagonist, with a view to further analyse the molecular profiles of skin inflammation. The obstacles encountered in the use of microdialysis were a long collection time, adsorption on to the polymeric materials used to construct the membranes and the outlet probe and the low analyte concentration in the extracellular fluid. The effect of the skin trauma after the insertion of the microdialysis catheter on the profile of mediators was also mentioned. Despite these limitations, she concluded that the levels of the mediators measured in the fluid extracted by microdialysis, skin suction blistering and microporation were the most representative of the overall levels mediators in the skin.

Among the other techniques for *in vivo* sampling of biomarkers include tape stripping, skin suction blistering, adsorption by sebutape, skin chamber technique, microporation, swabbing, scraping, transdermal analysis patch, skin surface wash sampling and skin biopsies. All methods except for microdialysis measure the total tissue concentration and require a correction factor to correct for tissue binding. Of note, microdialysis is the only technique currently available that is capable of selectively sampling the unbound pharmacologically active concentrations of the analyte in the tissue (46). This is an advantage as it reduces the scope for error.

Microdialysis has been applied in normal healthy skin to investigate cytokine and neuropeptide release in inflammation following exposure to thermal stimuli (40, 47). In their study from 2016, Quist et al. (47) showed 3 peaks with the release of T helper cells were detected in an early phase (4–12 h), intermediate phase (16–24 h) and late phases (32–40 h) post-exposure. The early-phase increase in cytokine levels was more TH1 dominated, and the late phase was more TH2 dominated, whereas in the intermediate phase, a mixed TH1/ TH2 response was detectable. In an earlier ultraviolet B (UVB) light study (40) microdialysis was performed 24 h before and after the UVB challenge. This study was stopped after 24 h, and lacked a negative control. Therefore, it could not be fully concluded that the observed increases in cytokines levels may have been a result UVB exposure as opposed to the repair processes experienced after tissue trauma post-catheter insertion.

Levels of calcitriol (vitamin D3) after UVB synthesis in healthy human skin (48) has also been assessed using microdialysis. The link between ultraviolet B irradiation and synthesis of calcitriol in the skin may be of immense importance for regulation of biological processes such as skin growth, differentiation, apoptosis and immunological reactions. This study demonstrates that photolysis of 7-dihydrocholesterol (7-DHC) induced by irradiation of human skin with UVB at 300 nm results in epidermal synthesis of calcitriol (vitamin D3). Cutaneous microdialysis was utilised as a valuable tool to mirror vitamin D3 metabolism in human skin.

The effects of drugs in healthy human skin have also been analysed through the use of microdialysis. The very first publication of this use was a study of ethanol absorption into the skin by Anderson et al. (49). The study demonstrated that ethanol does penetrate the skin and that maximum dermal levels varied from 15 to 800 µg/ml between subjects. However, ethanol was only sampled for 50 min which was subsequently concluded to be sub-optimal. Drugs that have been monitored using microdialysis in human skin include those on 8-methoxypsoralen (50), propranolol (51), methyl nicotinate (52), lidocaine (53), prilocaine (54), ethanol (49, 55), isopropranol (55), salicylic acid (56), nicotine (57), estradiol (58), diclofenac (13), metronidazole (59), tacrolimus (60), polyunsaturated fatty acids (60) and botulinum neurotoxin type A (BoNTA) (61).

A bioequivalence study where lidocaine delivery from two different vehicles was compared by dermal microdialysis sampling and pharmacodynamics assessment of the pain-relieving effect of the formulations was done previously (54). Thus, microdialysis has the potential to differentiate the magnitude of drug concentrations produced by different formulations. Silva et al. (61) provided the first evidence supporting the inhibitory effect of BoNTA on glutamate release in human skin. Two microdialysis catheters were inserted 1cm from the saline and BoNTA injections and diasylate was collected for 3 h. Glutamate concentrations were determined from this. An additional finding in this study using microdialysis was that BoNTA reduced capsaicin and mild heat-evoked pain intensity and skin blood flow. Through the effective use of microdialysis it was deduced that the blockade of glutamate release may be responsible for some of the analgesic action of BoNTA.

APPLICATIONS OF MICRODIALYSIS IN ATOPIC DERMATITIS

Further knowledge in to the pathophysiology of atopic dermatitis (AD) would be of immense value to this burdensome disease. Many studies have shown that as AD severity increases, quality of life decreases (62, 63). In

children, the International Study on Life with Atopic Eczema (ISOLATE) found major impacts of AD on self esteem; 27% of those had been teased or bullied because of AD and 36% said AD affected their self confidence (64).

Cutaneous microdialysis has been used in patients with AD to study the pathophysiology of AD, for example by measuring the concentrations of eicosanoids (60) and neuropeptides (65) in AD skin and comparing them with the concentrations found in healthy skin. In a similar fashion to its applications in healthy skin, it has also been used to analyse the effects and penetration of topical drugs (66, 67) on atopic skin as well as a tool to deliver drugs (68).

A recent study demonstrated through dermal microdialysis that levels of pro-inflammatory eicosanoids (prostaglandins and isoprostanes) are increased in lesional AD skin compared with nonlesional AD skin (62). In addition, treatment with the topical immune modulator tacrolimus suppresses the interstitial release of eisosanoids, thus reducing inflammation and subsequent skin erythema. Topical treatment with w-6-fatty acids was also found to reduce the level of isoprostanes, but not prostaglandin PGE2.

Microdialysis can be used to measure levels of neuropeptides in the skin. One study compared the levels in nerve growth factor (NGF) in atopic skin and healthy skin (65). It was previously reported that increased plasma levels of NGF and substance P in AD patients correlated with disease severity and plasma NGF and thereby was suggested as a marker of disease severity. This microdialysis study did not support this concept as it found that levels of NGF vary throughout the day and overall serum NGF levels were found to be lower in comparison to healthy subjects.

Microdialysis is also utilised as a tool for delivering drugs. Neisius et al. (68) applied prostaglandin E2 via microdialysis capillaries and analysed protein extravasation. The study did not reveal any significant difference ($p < 0.05$) in the time course of protein concentration between AD skin and controls. Thus, PGE2 was found to be a potent vasodilator and weak pruritic in both normal skin and atopic skin, yielding no evidence for a special use of PGE2 in AD.

Rudwied et al. (69) also used microdialysis capillaries to apply the mast cell degranulating substance compound 48/80 or histamine and also to deliver H1 blockade (cetirizine). Protein extravasation induced by the substance 48/80 and histamine was significantly reduced in AD patients. In addition, pruritus in AD patients was unchanged after H1 blockade, suggesting that mast cell mediators other than histamine are involved in itch in these patients.

Few techniques are available for the assessment of *in vivo* topical drug penetration in human skin. Dermal microdialysis is regarded as the technique of choice for the study of topical drug pharmacodynamics in AD skin. The penetration of topical metronidazole (59) and topical salicylic acid (56) have been assessed. Dermal microdialysis demonstrated a 2.4-fold increased penetration of the metronidazole topical formulation in active AD skin compared with uninvolved skin. Benfeldt et al. (56) demonstrated that the penetration of salicylic acid is initially much increased in AD skin compared with healthy skin but is reduced in skin with chronic AD. The reason for this is that the barrier capacity of the corneum stratum, (as measured by trans-epidermal water loss) in AD decreases with each topical treatment of salicylic acid. Thus, more skin barrier perturbation resulted in less drug penetration.

APPLICATIONS OF MICRODIALYSIS IN PSORIASIS

Variability in patient response to any given drug is still poorly understood. Even when a treatment response is seen, the degree of response is variable among patients (70). Cutaneous microdialysis could facilitate in filling gaps in knowledge in psoriasis. This would be of immense importance given the marked impact this disease has on a patient's physical and psychological quality of life. Clinical depression (13.8% vs 4.3%), anxiety (22.7% vs 11.1%) and suicidal ideation (17.3% vs 8.3%) are significantly higher in this cohort of patients compared with controls (71). Indeed, some psoriasis scoring tools incorporate psychosocial disability in to their assessment, emphasizing the psychological impact of this condition (72). The Salford Psoriasis Index is derived from combining a score of current severity of psoriasis based on the clinical assessment from the PASI, a score indicating psychosocial disability and a score based on historical information. This score recognises that some patients have a large degree of psychological disability from psoriasis despite a low PASI score.

Microdialysis has been used to measure endogenous substances in psoriatic skin. Sjögren et al. (73) carried out microdialysis on lesional and non-lesional psoriatic skin over 24 h in 3 subjects. These were compared to a reference group of 10 healthy controls. The median concentrations of 5 pro-inflammatory cytokines were collected hourly over 24 h and compared to controls. All microdialysis samples were investigated for cytokine content with a bead-based multiplex immunoassay from luminex. This study suggests that analysis of a 'cytokine fingerprint' for a range of cytokines or other mediators over 24 h is a possible basis for consideration in inflammatory dermatoses. The findings of note include a statistically significant elevation in the cytokine granulocyte-macrophage colony-stimulating factor in both lesional and non lesional skin of psoriatic patients at 3–8 h compared with controls ($p < 0.05$). In addition, IL-8 was found to be significantly elevated ($p < 0.05$) in the lesional psoriatic group compared with the controls at 0–2 h.

Another study assessed the cytokines profile of 3 patients receiving treatment with fumaric acid esters for 12 weeks (74). All patients showed significant clinical improvement and this correlated with a decrease in concentrations of the pro-inflammatory cytokines IL-6, IL-18, IL-23, but not IL-2 and adiponectin in lesional skin.

Besides cytokines, the insulin antagonizing adipokine resistin was found to be substantially elevated in psoriatic plaques in this study, indicating a metabolic state of insulin resistance (74). The presence of mediators inhibiting insulin receptor signalling in lesional psoriatic skin may directly contribute to the altered epidermal homeostasis characteristic for this disease. It is well established that insulin plays a major role in homeostasis of the skin (75).

IL-1β levels were monitored successfully using microdialysis (76). This cytokine is present in high concentrations in tissue fluid from patients with psoriasis and is reduced with successful anti-psoriatic therapy. It is already established that insulin is a pre-requisite for proper formation of the epidermal structure by participating in keratinocyte formation. IL-1β interferes with this mechanism and contributes to the pathogenesis of psoriasis with dual effects. Firstly, it blocks insulin-mediated differentiation by means of insulin resistance via p38MAPK (mitogen activated protein kinase). Secondly, IL-1β activates protein kinase B which induces proliferation and induces proliferation of keratinocytes. This study demonstrated the extent to which ILβ contributes to psoriasis via microdialysis and subsequent fluorescent bead immunoassay and was confirmed by immunohistochemistry of keratinocytes.

Microdialysis has been utilised for the investigation of granulocytes and histamine concentrations in psoriasis and to follow these parameters during therapy with high dose ranitidine (77). Compared to control skin in healthy volunteers, this study showed that mast cells are higher in number ($p < 0.05$), skin and plasma histamine concentrations are increased and histamine release to mast cell secretagogues (codeine and substance P) were raised by 10- and 2-fold, respectively at baseline. After treatment with a H2 antihistamine, ranitidine, these responses returned to normal. These results suggest a decrease in histamine release during remission in psoriatic patients. An earlier microdialysis study (78) echos these findings. Both the perfusion and interstitial concentrations of histamine as well as the net release of histamine after the topical application of capsaicin were significantly increased in both lesional and peri lesional skin.

Levels of iron and ascorbic acid in psoriatic skin have also been assessed using microdialysis (79). It has been shown that levels of free iron in these patients is significantly higher and the level of ascorbic acid significantly lower compared to healthy controls. It is hypothesised that the ascorbic acid is lower as a result of the inflammatory process in which free iron is released from storage proteins and then produces reactive oxygen species, which are then scavenged by anti-oxidants such as ascorbic acid.

Microdialysis is a useful technique for measuring drug levels in the psoriatic skin. Methotrexate levels have been assessed (80) and it was found that drug levels and the bioavailability of methotrexate was higher in lesional compared to nonlesional skin. Methotrexate levels reach a higher concentration at an earlier time point with a higher overall bioavailability but reside for shorter time in the psoriatic plaque than in the nonlesional skin. However, the pharmacokinetic profiles of patients receiving methotrexate are highly individual and not primarily dependent on dose or route of administration. The study demonstrated that subcutaneous administration was not superior to oral administration regarding bioavailability and drug levels or area under the curve, as other current studies suggest.

REFERENCES

1. Anderson CD. Cutaneous microdialysis: is it worth the sweat? J Invest Dermatol 2006; 126: 1207–1209.
2. Bito L, Davson H, Levin E, Murray M, Snider N. The concentrations of free amino acids and other electrolytes in cerebrospinal fluid, in vivo dialysate of brain, and blood plasma of the dog. J Neurochem 1966; 13: 1057–1067.
3. Ungerstedt U, Pycock CH. Functional correlates of dopamine neurotransmission. Bull Schweiz Akad Med Wiss 1974; 31–33: 44.
4. Lonnroth P, Jansson PA, Smith U. A microdialysis method allowing characterization of intercellular water space in humans. Am J Physiol Endocrinol Metab 1987; 253: E228–231.
5. Bolincier J, Ungerstedt U, Arner P. Microdialysis measurement of the absolute glucose concentration in subcutaneous adipose tissue allowing glucose monitoring in diabetic patients. Diabetologia 1992; 35: 1177–1180.
6. Bolinder J, Ungerstedt U, Arner P. Long-term continuous glucose monitoring with microdialysis in ambulatory insulin-dependent diabetic patients. Lancet 1993; 342: 1080–1085.
7. Persson L, Hillered L. Chemical monitoring of neurosurgical intensive care patients using intracerebral microdialysis. J Neurosurg 1992; 76: 72–80.
8. Müller M, Burgdorff T, Jansen B, Singer EA, Agneter E, Dorner G, et al. In vivo drug-response measurements in target tissues by microdialysis. Clin Pharmacol Ther 1997; 62: 165–170.
9. Globus MY, Busto R, Dietrich WD, Martinez E, Valdes I, Ginsberg MD. Effect of ischemia on the in vivo release of striatal dopamine, glutamate, and γ-aminobutyric acid studied by intracerebral microdialysis. J Neurochem 1988; 51: 1455–1464.
10. Fryk E, Sundelin JP, Strindberg L, Pereira MJ, Federici M, Marx N, et al. Microdialysis and proteomics of subcutaneous interstitial fluid reveals increased galectin-1 in type 2 diabetes patients. Metabolism 2016; 65: 998–1006.
11. Kennergren C, Mantovani V, Lönnroth P, Nyström B, Berglin E, Hamberger A. Monitoring of extracellular aspartate aminotransferase and troponin T by microdialysis during and after cardioplegic heart arrest. Cardiology 1999; 92: 162–170.
12. Müller M, Mascher H, Kikuta C, Schäfer S, Brunner M, Dorner G, Eichler HG. Diclofenac concentrations in defined tissue layers after topical administration. Clin Pharmacol Ther 1997; 62: 293–299.
13. Benfeldt E, Hansen SH, Vølund A, Menné T, Shah VP. Bioequivalence of topical formulations in humans: evaluation by dermal microdialysis sampling and the dermatopharmacokinetic method. J Invest Dermatol 2007; 127: 170–178.
14. Sjögren F, Anderson CD. Are cutaneous microdialysis cyto-

kine findings supported by end point biopsy immunohistochemistry findings? AAPS J 2010; 12: 741–749.
15. Groth L. Cutaneous microdialysis. Methodology and validation. Acta Derm Venereol 1996; Suppl 197: 1–61.
16. Clough GF. Microdialysis of large molecules. AAPS J 2005; 7: E686–692.
17. Sun L, Stenken JA. Improving microdialysis extraction efficiency of lipophilic eicosanoids. J Pharm Biomed Anal 2003; 33: 1059–1071.
18. Melgaard L, Hersini KJ, Gazerani P, Petersen LJ. Retrodialysis: a review of experimental and clinical applications of reverse microdialysis in the skin. Skin Pharmacol Physiol 2013; 26: 160–174.
19. Lonnroth P, Jansson PA, Fredholm BB, Smith U. Microdialysis of intercellular adenosine concentration in subcutaneous tissue in humans. Am J Physiol Endocrinol Metab 1989; 256: E250–E255.
20. Nielsen PN, Skov PS, Poulsen LK, Schmelz M, Petersen LJ. Cetirizine inhibits skin reactions but not mediator release in immediate and developing late-phase allergic cutaneous reactions. A double-blind, placebo-controlled study. Clin Exp Allergy 2001; 31: 1378–1384.
21. Song Y, Lunte CE. Calibration methods for microdialysis sampling in vivo: muscle and adipose tissue. Anal Chim Acta 1999; 400: 143–152.
22. Anderzhanova E, Wotjak CT. Brain microdialysis and its applications in experimental neurochemistry. Cell Tissue Res 2013; 354: 27–39.
23. Winter CD, Iannotti F, Pringle AK, Trikkas C, Clough GF, Church MK. A microdialysis method for the recovery of IL-1β, IL-6 and nerve growth factor from human brain in vivo. J Neurosci Methods 2002; 119: 45–50.
24. Anderson C, Andersson T, Wårdell K. Changes in skin circulation after insertion of a microdialysis probe visualized by laser Doppler perfusion imaging. J Invest Dermatol 1994; 102: 807–811.
25. Gill C, Parkinson E, Church MK, Skipp P, Scott D, White AJ, et al. A qualitative and quantitative proteomic study of human microdialysate and the cutaneous response to injury. J AAPS 2011; 13: 309–317.
26. Maischak H, Tautkus B, Kreusch S, Rhode H. Proteomic sample preparation by microdialysis: easy, speedy, and nonselective. Anal Biochem 2012; 424: 184–186.
27. De Lange EC, De Boer AG, Breimer DD. Methodological issues in microdialysis sampling for pharmacokinetic studies. Adv Drug Deliv Rev 2000; 45: 125–148.
28. Clough GF, Boutsiouki P, Church MK, Michel CC. Effects of blood flow on the in vivo recovery of a small diffusible molecule by microdialysis in human skin. J Pharmacol Exp Ther 2002; 302: 681–686.
29. Kirbs C, Kloft C. In vitro microdialysis recovery and delivery investigation of cytokines as prerequisite for potential biomarker profiling. Eur J Pharm Sci 2014; 57: 48–59.
30. Fellows PJ, Noble MR, Clough GF. Effect of perfusion rate on the recovery of albumin by microdialysis. J Vasc Res 2003; 40: 304–305.
31. Rosenbloom AJ, Ferris R, Sipe DM, Riddler SA, Connolly NC, Abe K, Whiteside TL. In vitro and in vivo protein sampling by combined microdialysis and ultrafiltration. J Immunol Methods 2006; 309: 55–68.
32. Trickler WJ, Miller DW. Use of osmotic agents in microdialysis studies to improve the recovery of macromolecules. J Pharm Sci 2003; 92: 1419–1427.
33. Erdő F, Hashimoto N, Karvaly G, Nakamichi N, Kato Y. Critical evaluation and methodological positioning of the transdermal microdialysis technique. A review. J Control Release 2016; 233: 147–161.
34. Joshi A, Patel H, Joshi A, Stagni G. Pharmacokinetic applications of cutaneous microdialysis: Continuous+ intermittent vs continuous-only sampling. J Pharmacol Toxicol Methods 2017; 83: 16–20.
35. Simmel F, Kirbs C, Erdogan Z, Lackner E, Zeitlinger M, Kloft C. Pilot investigation on long-term subcutaneous microdialysis: proof of principle in humans. J AAPS 2013; 15: 95–103.
36. de Lange EC. Recovery and calibration techniques: toward quantitative microdialysis. In Microdialysis in drug development. Springer, New York, NY, 2013; pp. 13–33.
37. Olson RJ, Justice JB. Quantitative microdialysis under transient conditions. Anal Chem 1993; 65: 1017–1022.
38. Weidner C, Klede M, Rukwied R, Lischetzki G, Neisius U, Schmelz M, et al. Acute effects of substance P and calcitonin gene-related peptide in human skin–a microdialysis study. J Invest Dermatol 2000; 115: 1015–1020.
39. Schmelz M, Luz O, Averbeck B, Bickel A. Plasma extravasation and neuropeptide release in human skin as measured by intradermal microdialysis. Neurosci Lett 1997; 230: 117–120.
40. Averbeck M, Beilharz S, Bauer M, Gebhardt C, Hartmann A, Hochleitner K, et al. In situ profiling and quantification of cytokines released during ultraviolet B-induced inflammation by combining dermal microdialysis and protein microarrays. Exp Dermatol 2006; 15: 447–454.
41. Eberle T, Doganci B, Krämer H, Fechir M, Wagner I, Sommer C, Birklein F. Mechanical but not painful electrical stimuli trigger TNF alpha release in human skin. Exp Neurol 2010; 221: 246–250.
42. Brooke RC, Sidhu M, Sinha A, Watson RE, Friedmann PS, Clough GF, Rhodes LE. Prostaglandin E2 and nitric oxide mediate the acute inflammatory (erythemal) response to topical 5-aminolaevulinic acid photodynamic therapy in human skin. Br J Dermatol 2013; 169: 645–652.
43. Rhodes LE, Belgi G, Parslew R, McLoughlin L, Clough GF, Friedmann PS. Ultraviolet-B-induced erythema is mediated by nitric oxide and prostaglandin E2 in combination. J Invest Dermatol 2001; 117: 880–885.
44. Brooke RC, Sinha A, Sidhu MK, Watson RE, Church MK, Friedmann PS, et al. Histamine is released following aminolevulinic acid-photodynamic therapy of human skin and mediates an aminolevulinic acid dose-related immediate inflammatory response. J Invest Dermatol 2006; 126: 2296–2301.
45. Falcone D, Spee P, Van De Kerkhof P, Van Erp PE. Minimally-invasive sampling of interleukin-1α and interleukin-1 receptor antagonist from the skin: A systematic review of in vivo studies in humans. Acta Derm Venereol 2017; 97: 1066–1073.
46. Erdő F. Microdialysis techniques in pharmacokinetic and biomarker studies. Past, present and future directions. A review. Clin Exp Pharmacol 2015; 5: 4.
47. Quist SR, Wiswedel I, Quist J, Gollnick HP. Kinetic profile of inflammation markers in human skin in vivo following exposure to ultraviolet B indicates synchronic release of cytokines and prostanoids. Acta Derm Venereol 2016; 96: 911–917.
48. Lehmann B, Sauter W, Knuschke P, Dressler S, Meurer M. Demonstration of UVB-induced synthesis of 1α, 25-dihydroxyvitamin D 3 (calcitriol) in human skin by microdialysis Arch Dermatol Res 2003; 295: 24–28.
49. Anderson C, Andersson T, Molander M. Ethanol absorption across human skin measured by in vivo microdialysis technique. Acta Derm Venereol 1991; 71: 389–393.
50. Tegeder I, Bräutigam L, Podda M, Meier S, Kaufmann R, Geisslinger G, et al. Time course of 8-methoxypsoralen concentrations in skin and plasma after topical (bath and cream) and oral administration of 8-methoxypsoralen. Clin Pharmacol Ther 2002; 71: 153–161.
51. Stagni G, O'Donnell D, Liu YJ, Kellogg Jr DL, Morgan T, Shepherd AM. Intradermal microdialysis: : Kinetics of iontophoretically delivered propranolol in forearm dermis. J Control Release 2000; 63: 331–339.
52. Boelsma E, Anderson C, Karlsson AM, Ponec M. Microdialysis technique as a method to study the percutaneous penetration of methyl nicotinate through excised human skin, reconstructed epidermis, and human skin in vivo. J Pharm Res 2000; 17: 141–147.
53. Kreilgaard M, Kemme MJ, Burggraaf J, Schoemaker RC, Cohen AF. Influence of a microemulsion vehicle on cutaneous bioequivalence of a lipophilic model drug assessed by microdialysis and pharmacodynamics. J Pharm Res 2001; 18: 593–599.
54. Anderson C, Andersson T, Nyquist-Mayer A, Melin M, Roberts MS. Cutaneous microdialysis technique in the study of the

percutaneous absorption of a local anaesthetic cream in vivo in human skin. In: Brain KR, James VJ and Walters KA, editors. Perspectives in Percutaneous Penetration. Vol. 5. STS Publishing; Cardiff. 1997, p. 46.
55. Benfeldt E, Serup J, Menne T. Effect of barrier perturbation on cutaneous salicylic acid penetration in human skin: in vivo pharmacokinetics using microdialysis and non-invasive quantification of barrier function. Br J Dermatol 1999; 140: 739–748.
56. Hegemann L, Forstinger C, Partsch B, Lagler I, Krotz S, Wolff K. Microdialysis in cutaneous pharmacology: kinetic analysis of transdermally delivered nicotine. J Invest Dermatol 1995; 104: 839–843.
57. Müller M, Schmid R, Wagner O, Osten BV, Shayganfar H, Eichler HG. In vivo characterization of transdermal drug transport by microdialysis. J Control Release 1995; 37: 49–57.
58. Miron DS, Rădulescu FS, Benfeldt E, Shah VP, Voicu VA. In vitro and in vivo evaluation of three metronidazole topical products. Pharm Dev Technol 2014; 19: 194–199.
59. Quist SR, Wiswedel I, Doering I, Quist J, Gollnick HP. Effects of topical tacrolimus and polyunsaturated fatty acids on in vivo release of eicosanoids in atopic dermatitis during dermal microdialysis. Acta Derm Venereol 2016; 96: 905–910.
60. Silva LB, Karshenas A, Bach FW, Rasmussen S, Arendt-Nielsen L, Gazerani P. Blockade of glutamate release by botulinum neurotoxin type A in humans: A dermal microdialysis study. Pain Res Manag 2014; 19: 126–132.
61. Alzolibani AA. Impact of atopic dermatitis on the quality of life of Saudi children. Saudi Med J 2014; 35: 391–396.
62. Ben-Gashir MA, Seed PT, Hay RJ. Quality of life and disease severity are correlated in children with atopic dermatitis. Br J Dermatol 2004; 150: 284–290.
63. Zuberbier T, Orlow SJ, Paller AS, Taïeb A, Allen R, Hernanz-Hermosa JM, et al. Patient perspectives on the management of atopic dermatitis. J Allergy Clin Immunol 2006; 118: 226–232.
64. Papoiu AD, Wang H, Nattkemper L, Tey HL, Ishiuji Y, Chan YH, et al. A study of serum concentrations and dermal levels of NGF in atopic dermatitis and healthy subjects. Neuropeptides 2011; 45: 417–422.
65. Ortiz PG, Hansen SH, Shah VP, Menné T, Benfeldt E. Impact of adult atopic dermatitis on topical drug penetration: assessment by cutaneous microdialysis and tape stripping. Acta Derm Venereol 2009; 89: 33–38.
66. Turpeinen M, Mashkilleyson N, Björkstén F, Salo OP. Percutaneous absorption of hydrocortisone during exacerbation and remission of atopic dermatitis in adults. Acta Derm Venereol 1988; 68: 331–335.
67. Neisius U, Olssonb R, Rukwied R, Lischetzki G, Schmelz M. Prostaglandin E2 induces vasodilation and pruritus, but no protein extravasation in atopic dermatitis and controls. J Am Acad Dermatol 2002; 47: 28–32.
68. Rukwied R, Lischetzki G, McGlone F, Heyer G, Schmelz, M. Mast cell mediators other than histamine induce pruritus in atopic dermatitis patients: a dermal microdialysis study. Br J Dermatol 2002; 142: 1114–1120.
69. Mrowietz U, Kragballe K, Reich K, Spuls P, Griffiths CE, Nast A, et al. Definition of treatment goals for moderate to severe psoriasis: a European consensus. Arch Dermatol Res 2011; 303: 1–10.
70. Dalgard FJ, Gieler U, Tomas-Aragones L, Lien L, Poot F, Jemec GB, et al. The psychological burden of skin diseases: a cross-sectional multicenter study among dermatological out-patients in 13 European countries. J Invest Dermatol 2015; 135: 984–991.
71. Kirby B, Fortune DG, Bhushan M, Chalmers RJ, Griffiths CE. The Salford Psoriasis Index: an holistic measure of psoriasis severity. Br J Dermatol 2000; 142: 728–732.
72. Sjögren F, Davidsson K, Sjöström M, Anderson CD. Cutaneous microdialysis: cytokine evidence for altered innate reactivity in the skin of psoriasis patients? J AAPS 2012; 14: 187–195.
73. Salgo R, Thaçi D, Boehncke S, Diehl S, Hofmann M, Boehncke WH. Microdialysis documents changes in the micromilieu of psoriatic plaques under continuous systemic therapy. Exp Dermatol 2011; 20: 130–133.
74. Boehncke S, Thaci D, Beschmann H, Ludwig RJ, Ackermann H, Badenhoop K, Boehncke WH. Psoriasis patients show signs of insulin resistance. Br J Dermatol 2007; 157: 1249–1251.
75. Buerger C, Richter B, Woth K, Salgo R, Malisiewicz B, Diehl S, et al. Interleukin-1β interferes with epidermal homeostasis through induction of insulin resistance: implications for psoriasis pathogenesis. J Invest Dermatol 2012; 132: 2206–2214.
76. Petersen LI, Hansen U, Kristensen JK, Nielsen H, Skov PS, Nielsen HJ. Studies on mast cells and histamine release in psoriasis: the effect of ranitidine. Acta Derm Venereol 1998; 78: 190–193.
77. Krogstad AL, Lönnroth P, Larson G, Wallin BG. Capsaicin treatment induces histamine release and perfusion changes in psoriatic skin. Br J Dermatol 1999; 141: 87–93.
78. Leveque N, Robin S, Muret P, Mac-Mary S, Makki S, Berthelot A, et al. In vivo assessment of iron and ascorbic acid in psoriatic dermis. Acta Derm Venereol 2004; 84: 2–5.
79. Quist SR, Quist J, Birkenmaier J, Stauch T, Gollnick HP. Pharmacokinetic profile of methotrexate in psoriatic skin via the oral or subcutaneous route using dermal microdialysis showing higher methotrexate bioavailability in psoriasis plaques than in non-lesional skin. J Eur Acad Dermatol Venereol 2016; 30: 1537–1543.