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Assessment of cutaneous drug delivery using microdialysis

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Abstract

During the last decade microdialysis has been successfully applied to assess cutaneous drug delivery of numerous substances, indicating the large potential for bioequivalence/bioavailability evaluation of topical formulations. The technique has been shown to be minimally invasive and supply pharmacokinetic information directly in the target organ for cutaneous drug delivery with high temporal resolution without further intervention with the tissue after implantation. However, there are a few challenges that need to be addressed before microdialysis can be regarded as a generally applicable routine technique for cutaneous drug delivery assessments. Firstly, the technique is currently not suitable for sampling of highly lipophilic compounds and, secondly, more studies are desirable for elucidation of the variables associated with the technique to increase reproducibility. The present literature indicates that the condition of the skin at the individual assessment sites is the main variable, but also variables associated with relative recovery, differentiation between the pharmacokinetic parameters (i.e., lag time, distribution, absorption and elimination rate) can influences the reproducibility of the technique. Furthermore, it has been indicated that cutaneous microdialysis in rats may be useful for prediction of dermal pharmacokinetic properties of novel drugs/topical formulations in man.

Keywords: Microdialysis; Cutaneous drug delivery; Bioequivalence; Bioavailability; Skin; In vitro–vivo correlation; Relative recovery; Variability

Contents

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1. Introduction

During the recent decades the advantages of cutaneous and percutaneous drug delivery has gained increasing attention for novel drug formulations. The percutaneous route may be an attractive solution for systemic delivery of very potent drugs with low oral bioavailability, low systemic clearance and narrow therapeutic window, due to the avoidance of hepatic first-pass metabolism and potential of long-term controlled release. However, the greatest potential for the topical administration route is targeted drug delivery to the skin itself, where dramatically higher skin-to-plasma ratios can be obtained compared to systemic drug delivery, and thereby maintain therapeutically effective drug concentrations in the target organ without the risk of inducing side-effects due to high systemic exposure [1]. While the in vivo efficiency of a topical formulation for percutaneous drug delivery is trivial to quantify in terms of bioequivalence/bioavailability by measurement of plasma concentrations, routine methods for assessment of cutaneous drug delivery are still not well established.

The most prevalent method for estimation of cutaneous drug delivery is still diffusion through excised animal/human skin or artificial membranes in the classical two-compartment Franz-type diffusion cells [2]. Even though this in vitro system has proven to be a robust screening system for early qualitative prediction of bioequivalence/bioavailability, the method obviously have several limitations. Among the most critical is the lack of elimination routes in terms of the vascular system and viable metabolising enzymes, alterations in the stratum corneum structure due to water uptake, and that the method actually determines percutaneous permeation instead of cutaneous penetration.

To obtain clinically relevant information about pharmacokinetic profiles in the skin, in vivo techniques must be applied. Previously, tape stripping of the skin has been a frequent technique to assess cutaneous drug delivery. This implies removal of the stratum corneum cell layers by consecutive adhesion of tape pieces to the skin surface and stripping of the top cell layers. The technique is, therefore, rather invasive, only assesses the penetration of drug into the stratum corneum (which is usually not the therapeutic target of cutaneous drug delivery) and can only determine a single concentration–time point per administration site. Furthermore, indirect radiochemical methods, skin biopsies, pharmacodynamic methods and more rarely suction blisters (which are usually applied to assess skin drug levels, following systemic administration) have also been applied to estimate in vivo skin penetration.

During the last decade microdialysis has been shown, by an accelerating number of publications [1,3–12,12–23], to be a very promising technique for assessment of cutaneous drug delivery. The technique is continuously evolving, but as it is still in its infancy in the dermatological research area, there are still a few issues that need to be addressed, before it can be regarded as a generally applicable routine technique for cutaneous drug delivery assessments.

The aim of this paper is to review the application of the microdialysis technique to investigate cutaneous drug delivery in animals and humans with focus on the variables associated with the method

and the prediction of human cutaneous bioequivalence/bioavailability (in this paper used as local bioavailability in the skin) from animal studies.

2. Theory and principles of microdialysis

2.1. Principles of microdialysis

A microdialysis fibre consists of a semipermeable membrane forming a thin hollow 'tube' (typically 0.2–0.5 mm diameter), which functionally resembles a blood vessel. The fibre only allows passage of molecules with a volume smaller than the openings in the membrane (termed 'cut-off' value). Depending on the probe design, the fibre has one end connected to an afferent impermeable tube, which leads to a micropump, and the other end to an efferent sampling tube. The sampling tube should possess as small a dead volume as possible, to minimise concentration gradient diffusions of the sampled drug after dialysis. The most prevalent designs are the linear probe, which is presently not commercially available, but is simple and inexpensive to manufacture from artificial kidney fibres [16,24], and the commercially available concentric probe, which is presently fairly costly relative to the homemade version (Fig. 1). For cutaneous microdialysis, the probe is implanted in the dermis of the skin via a guide cannula (Fig. 2). The microdialysis fibre is slowly perfused (typically 0.1–5 μl/min) with a physiological solution, which equilibrates with the extracellular fluid (ECF) of the surrounding tissue, exchanging substances smaller than the cut-off value of the membrane during the passage through the fibre (Fig. 3). Entering the microdialysis fibre, the solution is termed perfusate, and following dialysis of sub-

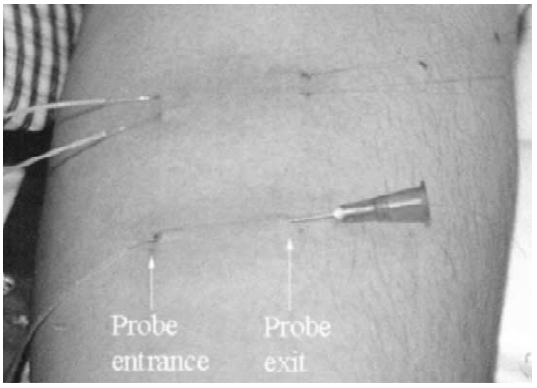

Fig. 2. Illustration of the implantation procedure of linear microdialysis probes via a guide cannula. Two probes have already been implanted and the guide cannula withdrawn in the upper part of the illustration (but have not yet been connected with efferent sampling tubes), and a third probe is being implanted via insertion through a guide cannula (bottom part of the illustration).

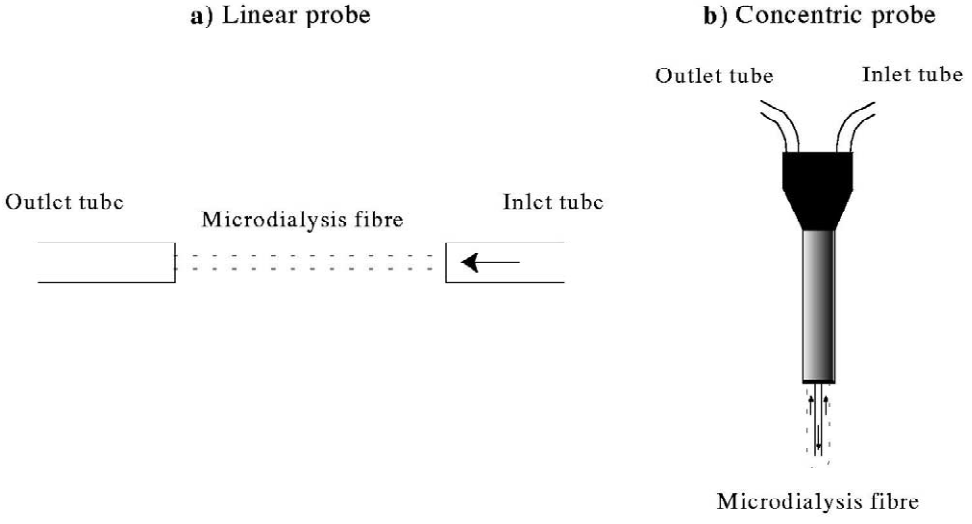

Fig. 1. Illustrations of the linear (a) and concentric (b) microdialysis probe design. Arrows indicate direction of perfusate flow.

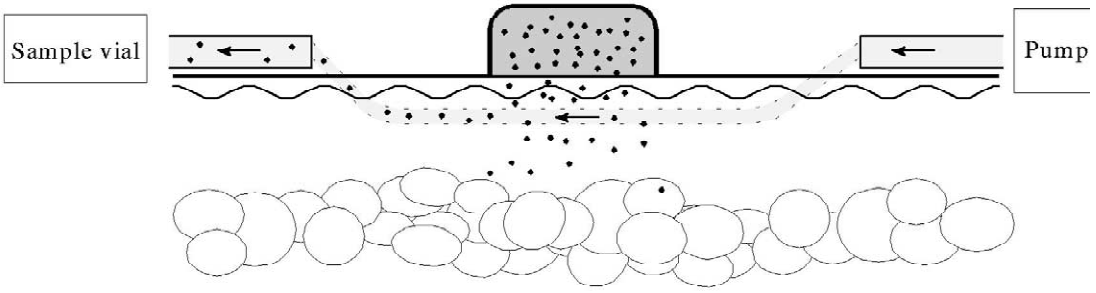

Fig. 3. Sampling of substances from a topical application with the microdialysis technique in the dermis of the skin.

stances, the solution exiting the fibre is termed dialysate.

The exchange of substances occurs due to concentration gradients according to Fick's second law of diffusion [25], and the speed of equilibrium is consequently proportional to the size of the gradient and diffusion rate of the substance in the medium, in addition to the surface area of the fibre membrane.

As substances are able to diffuse in both directions through the membrane, microdialysis can principally be used to both extract and deliver substances in the tissues.

2.2. Features of microdialysis

The main feature of microdialysis is the possibility of assessing substance levels directly in the target tissue, which is very useful for comparison of pharmacokinetic and pharmacodynamic responses. The technique enables estimation of both endogenous and exogenous substances in most tissues and organs, and may also be used for delivery of substances to tissues. Due to the typically low cut-off values of microdialysis membranes, samples are protein free and readily analysable without the need for further analytical purification. The free protein-unbound drug fraction is, therefore, determined directly. As the level of unbound drug generally corresponds with the pharmacodynamic response in the tissues, this feature further adds to the pharmacological relevance. Fibres with higher cut-off values (100–3000 kDa) are also available if macromolecule or protein sampling is the target [26].

Once probe implantation has been done, full pharmacokinetic profiles (up to several days) can be obtained from each sampling site without further intervention [27]. The pharmacokinetic profile can principally be of very high temporal resolution depending on the flow rate of the perfusate and the analytical method (online analytical systems have been established which continuously monitor the drug levels).

Furthermore, the technique is minimally invasive, and only implies a minor reversible trauma by insertion of the guide cannula used for the implantation of the microdialysis probe [16,28–31].

2.3. Recovery

The partition of a substance between the perfusate and the ECF depends on the composition of the perfusate and the hydrophilic/lipophilic properties of the ECF surrounding the microdialysis fibre. Furthermore, due to the short passage duration of the perfusate through the fibre, complete equilibration is often not attained between the perfusate and the ECF. The fraction of drug, which is collected in the dialysate, relative to the actual ECF concentration of unbound drug is termed relative recovery. The total amount of drug collected in the dialysate is defined as absolute recovery.

Relative recovery is theoretically independent of the compound concentration since the concentration gradient and partition coefficient is proportional to the amount, which diffuses into the perfusate. This is a prerequisite for application of the microdialysis technique to estimate true unbound extracellular levels of a compound. However, technical problems, e.g., adhesion of the compound of interest to the microdialysis probe, can render the concentration independency obsolete, and should hence be ex-

amined in vitro prior to onset of a microdialysis study [32].

The absolute recovery of a substance is a critical parameter for the success of the microdialysis technique. A low absolute recovery will proportionally diminish the sample concentrations and/or lead to unacceptable long sampling periods. In addition to the low sample volumes (typically 10–100 μl) obtained in microdialysis due to the low flow rate of the perfusate, this will stress the analytical method and require a very sensitive detector to enable quantification of the samples.

Drug recovery is affected by several parameters, which can be manipulated to increase drug content in the samples (Table 1).

Relative recovery increases with:

1. Larger surface area of the microdialysis membrane [32,33], which will increase the total area available for diffusion of the drug into the perfusate, and thereby accelerate the equilibrium process. If the drug is completely equilibrated between the perfusate and the ECF, an increase in surface area will not influence relative recovery.
2. Declining perfusate flow [32,34]. A lower perfusate flow will allow more time for the drug to enter the perfusate and, comparable to the increase in membrane surface area, increase the equilibrium process.
3. Higher temperature will also accelerate the equilibrium process, according to the standard laws of physics for diffusion [32,35].
4. Higher diffusivity of the drug in the surrounding tissues/ECF, i.e., local clearance in the vicinity of the probe. Substances with lower MV will diffuse faster in the tissue. The diffusion rate of a substance also depends on the structure, charge, and surface activity [36].
5. Higher affinity of the substance to the perfusate, which will increase the partition coefficient of drug between the perfusate and ECF. Depending on the physico-chemical properties of the drug, the composition/pH of the perfusate may be altered accordingly to increase the solubility of the drug, relative to that of the surrounding ECF.

Absolute recovery increases with:

1. The factors which increases relative recovery (potentially apart from decreasing flow rate).
2. Absolute unbound tissue concentrations of the substance, i.e., lower tissue clearance of the drug and protein-bound fraction of the substance [32].
3. Higher perfusate flow (?). If the corresponding decrease in drug concentration (relative recovery) does not exceed the proportional increase in total sampled drug amount by higher sample volume, higher absolute recovery will be obtained by increasing the perfusate flow.
4. More superficial probe implantation depth, relative to the application site [23]. When the stratum corneum constitutes the rate limiting barrier for cutaneous drug delivery and sink conditions are maintained in the deeper subcutaneous layers, a concentration gradient is formed after topical application of a compound (Fig. 4). However, the significance of this correlation on cutaneous microdialysis is questionable as the implantation range in the dermis typically varies between 0.5 and 1.0 mm, where the corresponding concentration gradient is not very pronounced for the majority of studied compounds [3,4,8,9,32].

Most of these parameters are optimised prior to

Table 1
Theoretical correlation between various factors influencing relative and absolute recovery of microdialysis probes relative to total free unbound drug concentration in the skin

Recovery	Membrane area	Perfusate flow	Temp.	Diffusivity in surrounding tissue	Perfusate solubility	Unbound free fraction in ECF	Implantation depth
Relative	↑	↓	↑	↑	↑	↔	↔
Absolute	↑	↓↑	↑	↑	↑	↑	↓

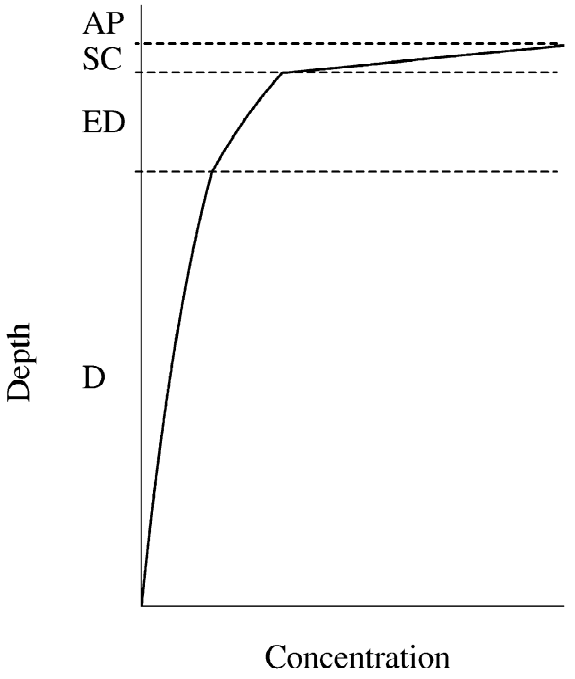

Fig. 4. Theoretical correlation between drug skin concentration and depth following topical application of a drug (AP), where the stratum corneum (SC) is the main diffusion barrier and sink conditions is maintained through drug elimination in the epidermal (ED) and dermal (D) layers.

onset of a microdialysis experiment, and can be regarded as constants, which do not induce variability in the study results. However, diverging inter- and intra-individual tissue properties, causes the relative recovery to differ between experiments [11,12,23] and may even be subject to changes within an experiment [11,12]. Relative recovery should therefore preferably be determined individually for each probe and, if possible, monitored during the experiment. The two main variables in the skin are clearance of the drug from the tissues surrounding the microdialysis membrane, which is affected by enzymatic activity and capillary blood supply (depending on the major route of elimination) and the partition coefficient between the tissues and the perfusate, which is affected by lipophilicity and pH of the surrounding tissues/ECF.

While exact estimation of unbound tissue concentrations is not crucial to bioequivalence studies, the method of recovery estimation should reliably reflect changes in relative recovery between microdialysis samples in order to quantify the relative difference between pharmacokinetic parameters. In order to enable comparisons between studies, and to correlate pharmacodynamic effects to the pharmacokinetic profile of a drug, emphasis should be made to obtain relative recovery values as close to true values as possible.

Several methods have been used to assess relative recovery in microdialysis experiments. Results from in vitro methods are often not reliable indicators for in vivo recovery [32]; however, these methods are often applied initially to ensure a concentration-independent relative recovery of the applied microdialysis equipment, and can provide a rough estimate of the magnitude of in vivo recovery. Among the in vivo methods, the most reliable methods are the ‘point of no net-flux’, which requires steady state drug concentrations and utilises the principle of diffusion of the substance from the perfusate into the tissue will occur for perfusate concentrations lower than the true tissue value and opposite for higher concentrations [24], and the retrodialysis method [37]. For extensive reviews of the various methods for recovery estimation, see Kehr [38], Parsons and Justice [33] and Ståhle [25].

The most prevalent experimental method for recovery estimation in cutaneous microdialysis in vivo is the retrodialysis method.

2.3.1. Retrodialysis

The retrodialysis method assesses the loss of the drug from the perfusate, and relies on the assumption that net drug transport, through the microdialysis membrane, from the perfusate into the surrounding tissues, equals the net drug transport from the tissues into the perfusate. Relative recovery (RR) is calculated as following:

$$\mathrm{RR} = \left(\frac{C_{\mathrm{perfusate}} - C_{\mathrm{dialysate}}}{C_{\mathrm{medium}} - C_{\mathrm{perfusate}}} \right) \quad (1)$$

A prerequisite for correct estimation of relative recovery with the retrodialysis method is therefore a negligible concentration of the compound in the medium (ECF) surrounding the probe (attainment of ‘sink’ conditions), which is generally assured by the rapid vascular clearance of substances from the dermis. The equation for calculation of relative

recovery with the retrodialysis method is hence often abbreviated to:

$$RR = \left(\frac{C_{perfusate} - C_{dialysate}}{C_{perfusate}} \right) \quad (2)$$

To date, (sub-)cutaneous microdialysis recovery values have typically been estimated by the retrodialysis method with the substance of interest in separate experiments [4,5,23], prior or subsequent to the experiment [8], which does not enable correction of interindividual differences in relative recovery. Additionally, when relative recovery varies during the experiment, this approach may lead to skewed corrected estimates of the true unbound tissue concentrations [11,12].

To monitor recovery during the experiment, retrodialysis by calibrator (also referred to as 'internal standard' or 'reference' method) has successfully been introduced to microdialysis in lung/blood [39], brain [40] and to monitor endogenous glucose in the skin [37,41–43]. Recently, this technique has also successfully been applied to cutaneous drug delivery studies [11,12]. The calibrator should have physicochemical and local pharmacokinetic properties that are similar to the drug to be a reliable indicator of changes in recovery level. If relative recovery of the calibrator differs significantly from the drug of interest, the ratio between the two should be determined subsequent or prior to the experiment by addition of both to the perfusate [40] and relative recovery of the drug during the experiment can be calculated according to:

$$RR = \left(\frac{C_{perfusate} - C_{dialysate}}{C_{perfusate}} \right) \times \left(\frac{RR_{drug}}{RR_{calibrator}} \right) \quad (3)$$

2.4. Invasiveness

The skin traumas induced by the probe implantation procedure, includes an increase in blood flow and erythema, wheal of the skin, and histamine release [28–31,44–46]. As drug recovery is very much affected by alterations in blood flow and other parameters which influences elimination, the minor trauma, which is inflicted by implantation of the microdialysis probe, should be diminished before onset of the experiment. Studies have shown that probe design, species and anaesthesia are important factors, which determines the duration of the trauma. Groth et al. studied skin traumas in both rats [29,46] and humans [30] following insertion of linear microdialysis probes with a 21-gauge (0.8 mm inner diameter (i.d.)) cannula. The studies showed an initial increase in blood flow, skin thickness and histamine levels in rats, which were normalised approximately 30 min after probe insertion. Skin wheal (30% of normal thickness) was, however, not significantly reduced during the experiments. Similar observations were made in humans; however, the vascular effects required a minimum of 90 min to normalise. Concurrent injection of lidocaine reduced the vascular effects of the trauma. Anderson et al. found a normalisation of the increased blood flow [28] in humans 60 min after insertion of a 0.5-mm outer diameter (o.d.) concentric probe, and a normalisation of the increased histamine levels [45] after 40 min. A decrease in vascular effect if the subjects received local anaesthesia (mepivacaine) prior to the insertion was also demonstrated by these studies. By initial application of EMLA cream (lidocaine, prilocaine), Petersen [31] observed a return to blood flow baseline levels after 40 min, following insertion of a linear probe with a 23-gauge guide cannula (0.6 mm i.d.) in humans. Generally it appears that smaller traumas develop from implantation of probes (or more correctly, guide cannula used for the implantation) with smaller diameter, and the increase in blood flow subsides faster in rats compared to humans and by concurrent application of local anaesthetics.

A histological study of the cell layers of rat skin following implantation of a linear probe (using a 25-gauge cannula) over 32 h, have indicated that no significant oedema or blood accumulation occurs around the probe after implantation [16]. However, infiltration of lymphocytes after 6 h and development of scar tissue after 24 h was observed. In another study, Ault et al. demonstrated an increase in transdermal flux in vitro of 5-fluorouracil when a concentric probe (via a 21-gauge cannula) was implanted [47] compared to a linear probe (via a 25-gauge cannula) [16], indicating that the tissue disruption was greater by insertion of a concentric probe. The observation was most likely due to the smaller diameter of the guide cannula used for insertion of the linear probe.

2.5. Current limitations and challenges

The microdialysis technique has been demonstrated to be applicable to multiple tissues and organs for sampling of numerous different substances. The most substantial challenges for the microdialysis technique today is sampling of lipophilic substances, due to the low relative recovery [32,48]. The current limitations for sampling of very lipophilic substances are related to the hydrophilic nature of the perfusate applied for most microdialysis experiments, and possibilities of adherence of the drug to the microdialysis equipment. Presently, an isotonic aqueous buffer, e.g., Ringers solution, is often used as perfusate, in which lipophilic substances have a very low solubility and hence low relative recovery. The low solubility of lipophilic substances can principally be solved by the addition of solvents (e.g., polyethylene glycol, cyclodextrins, lipids or proteins) to the perfusate [49–51], or by changing the pH of the perfusate if the substance is acidic or alkaline. However, considerations should be made to insure compatibility between the perfusate and the tissues surrounding the microdialysis fibre.

Previously, sampling of substances with high MV has also been considered a limitation for the microdialysis technique. However, with the recent introduction of microdialysis membranes with cut-off values around 3000 kDa [26,52], the challenge has instead shifted to the analytical methods where sample preparation may be required due to the subsequent introduction of macromolecules and proteins in the dialysate. This can be problematic with the small sample volumes typically collected (10–50 μl). On-line sample purification with, e.g., turbulent flow chromatography [53] or similar analytical methods, has diminished this challenge, though.

The current limitations and challenges of microdialysis sampling today with low relative recovery is, therefore, also very much related to analytical limitations. Past studies have mainly used regular and narrowbore HPLC with UV-detection to quantify substance levels in microdialysis samples, which may be adequate if relative recovery of the substance is high. However, these conventional analytical methods are often the limitation to the application of the microdialysis techniques for substances with low recovery [48]. Recent introduction of the microbore/capillary LC methods and more sensitive detectors, e.g., mass spectrometers, biosensors, etc., to analyse microdialysis samples are methods which has extensively broaden the range of substances that can be sampled and analysed by the microdialysis technique. The analytical aspects of microdialysis have been described in detail by Davies et al. [54].

3. Animal investigations

To date, all cutaneous drug delivery studies using microdialysis in animals have been performed in rats. An overview of the studies is presented in Table 2, and are described more detailed in the following section.

3.1. Single formulation studies

The feasibility of microdialysis to sample 5-fluorouracil in the skin has been demonstrated by Ault et al. [16]. Steady-state levels of the drug following topical application were determined in six awake fuzzy rats over a period of 12 h. A more than 10-fold difference in the observed C_{ss} (ranging from 0.033 ± 0.008 to 0.38 ± 0.30 μg/ml), illustrated the relative large variability of dermal levels of the penetrated drug assessed by the microdialysis technique.

Benfeldt and Serup [5] have studied the penetration of salicylic acid in hairless rats with the objective to investigate barrier function of the skin, following treatment by acetone, sodium lauryl sulphate and tape stripping, respectively. The AUCs of salicylic acid determined by microdialysis sampling in the dermis of the skin, correlated well with transepidermal water loss and erythema, indicating that microdialysis is an effective assessment technique for skin barrier function. Average relative recovery, determined by the retrodialysis method in separate experiments ($n = 11$), was $29 \pm 4\%$ and was indicated to be independent of anatomic region of the rat.

Following 3 days occluded application of high drug amounts, Benfeldt and Groth [48] attempted to sample fusidic acid ($\log P = 2.7$, 97% protein binding) and betamethasone-17-valerate ($\log P = 3.5$, low protein binding) in rats by the microdialysis tech-

Table 2
Overview of cutaneous drug delivery studies using microdialysis in animals

Drug	Probe	Perfusate	Perfusate flow (μl/min)	Species	RR (%)	Refs.
Enoxacin	Concentric, 4 mm	Ringer	1	Wistar rats	13–34	[13]
Diclofenac	Concentric, 10 mm	Ringer	1	Wistar rats	80–90	[14]
Ondansetron	Concentric, 10 mm (18 kDa)	Saline	1	Rats	33±1.8	[15]
5-Fluorouracil	Linear, 5×0.2 mm (9 kDa)	Ringer	2	Fuzzy rats	20–25	[16]
Fusidic acid	Linear, 30×0.2 mm (2 kDa)	Phosphate buffer pH 7.4, 2.5 mM glucose	2	Hairless rats	44[a]	[48]
Betamethasone-17-valerate	Linear, 30×0.2 mm (2 kDa)	Phosphate buffer pH 7.4, 2.5 mM glucose	2	Hairless rats	38[a]	[48]
Methotrexate	Concentric, 10×0.5 mm (20 kDa)	Ringer, pH 6.5	1	Wistar rats	n.d.	[17]
Valproate	Concentric, 10×0.5 mm (20 kDa)	Ringer, pH 6.5	1	Wistar rats	n.d.	[19]
Cyclosporin	Concentric, 10×0.5 mm (20 kDa)	Ringer, pH 6.5	1	Wistar rats	n.d.	[18]
Tranilast	Linear, 30×0.2 mm (2 kDa)	Tyrode	3	Wistar rats	n.d.	[7]
Salicylic acid	Linear, 30×0.2 mm (2 kDa)	Phosphate buffer pH 7.4, 2.5 mM glucose	5	Hairless rats	29±4	[5]
	Concentric, 4×0.5 mm (20 kDa)	Tyrode	3	Wistar rats	4–5[b]	[6]
Lidocaine	Linear, 30×0.2 mm (2 kDa)	Glucose-Ringer pH 6.5	1.2	Wistar rats	69–91	[11]
Prilocaine	Linear, 30×0.2 mm (2 kDa)	Glucose-Ringer pH 6.5	1.2	Wistar rats	58–98	[11]

n.d., not determined.
[a] Only determined in vitro.
[b] Determined relative to full skin.

nique, using a standard glucose-Ringer solution as perfusate. They failed to recover fusidic acid in the dialysate, and only collected very low sample concentrations of betamethasone-17-valerate. This study illustrated the present challenge of sampling highly lipophilic and protein-bound drugs in the traditional aqueous perfusate applied for most cutaneous microdialysis experiments today.

3.2. Bioequivalence/bioavailability studies

The first report of an in vivo cutaneous microdialysis study in animals was by Matsuyama et al. [17] in 1994, who performed a bioequivalence study with different levels of a penetration enhancer (1-[2-(decylthio)ethyl]azacyclopentan-2-one, (HPE-101)) applied to a topical vehicle. The authors successfully applied the microdialysis technique to differentiate between accumulating dialysed amounts of methotrexate over 10 h from the dermis of Wistar rats, according to the different vehicle levels of the enhancer. Matsuyama and co-workers have further published two studies concerning the enhancement of cutaneous drug delivery using various concentrations of HPE-101 in the formulations, using the microdialysis technique [18,19]. The studies showed that dermal levels of valproate and cyclosporin in rats could be significantly increased, up to 80 times for valproate and seven times for cyclosporin, by the addition of the enhancer to the basic topical vehicle. The dermal apparent absorption rates were assessed as slope of the linear part of accumulated amount of the drugs in the dialysate versus time curves. A linear curve of accumulated amount of drug versus time is only obtained at steady-state drug levels in the microdialysis samples (dermis), where the frac-

tional increase in accumulated amount is the same. The bioequivalence parameters is hence actually an assessment of C_{ss}, which is proportional to the absorption rate and inverse proportional to the elimination rate and volume of distribution, providing an average of these parameters.

A substantial increase in dermal tranilast levels, in terms of C_{max} and AUC, by topical application in a vehicle containing up to 20% oleic acid and 0–10% propylene glycol (PG) has also been demonstrated by use of the microdialysis technique [7]. The study demonstrated a good quantitative agreement between relative increase in C_{max} and AUC between the six formulations in the dermis and in plasma, respectively, indicating that the enhancers increased both dermal and transdermal delivery. The dermal levels (determined by AUC) were indicated to be more than 400 times higher following topical application, compared to intravenous injection of a similar dose tranilast. The study was an excellent demonstration of topical administration as the primary route of choice for future treatment of keloid and hypertrophic scars with tranilast.

Relative recovery in vivo was not estimated in any of the above-mentioned studies and the reported dermal concentrations of the drugs are, therefore, not reliable indicators of true unbound tissue concentrations and comparisons between different tissues, assessment techniques or studies should be done with caution. Nevertheless, these early studies indicated the tremendous potential of the microdialysis technique for bioequivalence/bioavailability studies to optimise topical vehicles for cutaneous drug delivery.

The enhancer effect of oleic acid on dermal delivery has also been demonstrated by Ding et al. [15], using ondensetron hydrochloride as model drug. Both 2 and 5% oleic acid in PG linearly increased relative steady-state delivery rates compared to neat PG, and also lag time was substantially reduced.

Murakami et al. [6] have published a study evaluating the effect of different topical vehicles on the dermal absorption of salicylic acid compared to systemic levels (transdermal). A 100-fold difference was observed between C_{max} and AUC of dermal levels of salicylic acid, with a w/o emulsion providing the most extensive cutaneous drug delivery and water-soluble solbase vehicle the least. The ratio between AUC_{dermal} and AUC_{plasma} diverged between the formulations, leading he authors to the assumption that several of the vehicles enabled retention of the drug in the skin, increasing dermal drug delivery relative to transdermal. These results emphasise the relevance of estimating cutaneous drug delivery in the dermis, which can currently only be done continuously in vivo by the microdialysis technique. Recovery of salicylic acid was determined as the concentration ratio between the dialysate (Tyrode solution) and excised skin of the sacrificed rat, and was estimated to be 4–5%. This is 6–7-fold lower, than that estimated by the retrodialysis method [5], which is presumably attributable to the distribution between unbound drug in the ECF and total skin concentrations.

Microdialysis has also recently been applied to assess cutaneous drug delivery using iontophoresis [13,14], for which the technique appears to be a promising tool, as iontophoresis is mainly used with ionised and highly polar molecules. Fang et al. have examined the influence of hydrogel formulations on dermal penetration of diclofenac [14] and enaxin [13], respectively. Two polymers (polyvinylpyrrolidone and hydroxypropyl methylcellulose) were used for the diclofenac hydrogels, either alone or combined, and the latter in addition to enhancer pretreatment with cardamom oil. Even though in vivo relative recovery of was high (80–90%), diclofenac was barely detectable in the dialysate when no enhancers were applied. Twelve hours pre-treatment with cardamom oil significantly increased dermal drug levels, and further elevation of dermal ECF levels was observed for all four formulations when iontophoresis was applied. In the latter study, the effect of pH and addition of Azone to the hydrogels on the iontophoretic delivery of enaxin to the skin was examined. Significant higher dermal drug levels could be demonstrated by use of unbuffered hydrogel or addition of Azone compared with a hydrogel buffered at pH 5.

The influence of topical microemulsion composition and internal structure on the cutaneous absorption of a lipophilic (lidocaine) and a hydrophilic model drug (prilocaine hydrochloride), respectively, has been investigated in Wistar rats [11]. By means of the retrodialysis by calibrator method a high relative recovery was found in vivo for both lidocaine (69–91%) and prilocaine (58–98%) using an

isotonic aqueous perfusate buffered at pH 6.5. To monitor relative recovery during the experiments, the study introduced the retrodialysis by calibrator method. Relative recovery was demonstrated to vary, not only between probes, but also within the individual probes during the experiment, generally decreasing slightly, which emphasised the relevance of monitoring recovery during the experiments. Furthermore, the microdialysis technique enabled demonstration of significant influence of microemulsion composition on the dermal drug delivery of both drugs.

4. Human investigations

An overview of the cutaneous drug delivery studies using microdialysis in humans is presented in Table 3. The studies are described more detailed in the following section.

4.1. Single formulation studies

The very first publication of a cutaneous microdialysis was a study of ethanol absorption into the skin by Anderson et al. [9]. The study demonstrated that ethanol does penetrate the skin and that maximum dermal levels varied from 15 to 800 μg/ml between subjects during the sampling period. Dialysate was, however, sampled for 50 min and only collected two times in five of the subjects, and one time in four of the subjects. In a later study, Anderson et al. [10] extended the study of percutaneous absorption of organic solvents sampled by microdialysis, to include isopropanol and by means of an increased temporal resolution (samples col-

Table 3
Overview of cutaneous drug delivery studies using microdialysis in humans

Drug	Probe	Perfusate	Perfusate flow (μl/min)	RR (%)	Refs.
8-Methoxypsoralen	Concentric, 30×0.6 mm (20 kDa)	Water	2	25[a]	[1]
Propranolol	Linear, 10×0.2 mm (20 kDa)	Ringer, lactate	3	53±14	[20]
Methyl nicotinate	Concentric, 10×0.5 mm (20 kDa)	Ringer	5/1	26/65[a]	[21]
Lidocaine	Linear, 30×0.2 mm (2 kDa)	Glucose-Ringer pH 6.5	1.2	56–95	[12]
	Concentric, 4×0.5 mm (20 kDa)	Ringer	3	n.d.	[22]
Prilocaine	Concentric, 4×0.5 mm (20 kDa)	Ringer	3	n.d.	[22]
Ethanol	Concentric, 10×0.5 mm (20 kDa)	Ringer	1	90	[9]
	Concentric, 10×0.5 mm (20 kDa)	Ringer	3	n.d.	[10]
Isopropanol	Concentric, 10×0.5 mm (20 kDa)	Ringer	3	n.d.	[10]
Salicylic acid	Linear, 30×0.2 mm (2 kDa)	Phosphate buffer pH 7.4, 2.5 mM glucose	5	24±4	[4]
Nicotine	Concentric, 10×0.5 mm (20 kDa)	Ringer	10	13[a]	[3]
	Concentric, 16×0.5 mm (20 kDa)	Ringer, 7% albumin	1.5	25–36	[23]
Estradiol	Concentric, 16×0.5 mm (20 kDa)	Ringer, 7% albumin	1.5	1–3	[23]
Diclofenac	Concentric, 16×0.5 mm (20 kDa)	Ringer	1.5	66±12	[8]

n.d., not determined.
[a] Determined in vitro.

lected every 10 min for 140 min), detectable levels of ethanol and isopropanol could be demonstrated in the dermis after 20 min in all subjects and appeared to reach a plateau after 100 min. The research group has also published a preliminary report, which demonstrated that lidocaine and prilocaine can be sampled from the dermis by the microdialysis technique, following topical application of EMLA [22].

Comparable to their studies in rats, Benfeldt et al. [4] have investigated the cutaneous penetration of salicylic acid through normal and perturbed skin in humans. The average relative recovery of the drug was determined in two individuals (two probes each), independently of the experiments, and was assessed to be 24±4%, which differed substantially from the determined in vitro recovery (80±3%), illustrating the unreliability of basing in vivo recovery on in vitro results. Salicylic acid could be detected in the dialysate, already 10 min after application in both normal and perturbed skin, indicating a very short lag time of the drug, and the cutaneous penetration of the drug was substantially increased, in terms of AUC of the concentration–time curves in the skin, with increasing barrier perturbation. The microdialysis technique was demonstrated to be substantially more sensitive than transepidermal water loss and erythema to assess barrier function of the skin by weak barrier perturbations. Penetration was assessed in four different locations on the left volar forearm, equally distanced from the elbow to the hand. No intraregional variation in barrier function of the skin was observed.

The feasibility of microdialysis to study iontophoretic drug delivery in humans has been demonstrated with propranolol as model drug [20].

The pharmacokinetic profile of nicotine in the dermis following application of a commercially available patch (Nicotinell TTS 20, Ciba-Geigy), has been reported by Hegemann et al. [3]. Detectable levels of the drug in the microdialysis samples were demonstrated approximately 120–150 min after application of the patch in nine subjects, and reached a plateau within 330–360 min. However, large interindividual variability of C_{max} was observed, ranging from 500 to 2140 ng/ml. In vivo recovery was not determined.

Nicotine levels in defined cutaneous/subcutaneous tissue layers following application of a transdermal patch (Nicotinell TTS 30, Ciba-Geigy (21 mg/24 h)), has additionally been investigated by Müller et al. [23]. Microdialysis probes where implanted 2–9 mm beneath the skin surface, and nicotine was detectable in all skin layers. A correlation was found between AUC of concentration–time curves, C_{ss} and probe depth. This correlation was even higher by day-to-day assessments in a single individual, indicating interindividual variability to be largest. It was also attempted to investigate the transdermal permeation of estradiol from a patch (Estraderm TTS 100 μg/24 h); however, this failed to recover the drug in detectable amounts in the dialysate with probe depths ranging from 1.5 to 10 mm. In vivo relative recovery was estimated on separate study days by the retrodialysis method and ranged from 25 to 36% for nicotine and 1 to 3% for estradiol.

Dermal methyl nicotinate could only be detected in two out of three subjects when a high dose (100 mM) was applied topically for 10 min, even though in vitro studies indicated a high relative recovery (66% at 1 μl/min flow) [21]. When the formulation was applied for 1 min, nothing could be detected in five subjects. Fast absorption and elimination was demonstrated in excised skin in vitro using microdialysis with the same application periods, but the in vivo study may have been hampered by the short application periods, rapid degradation of the drug in the skin in conjugation with elevated capillary blood flow due to extensive development of erythema/edema at the application site. Also, in vivo recovery, which was not determined, could have been much lower than that indicated in vitro.

Transdermal delivery of diclofenac in subcutaneous tissues has additionally been investigated by Müller et al. [8]. Probes were placed in two defined tissue layers (3.9±0.3 mm and 9.3±0.5 mm). Diclofenac was only detected in the dialysate (Ringer's solution) in 11 out of 20 subjects, independently of the probe depth, and the average AUC of the concentration–time curves from the two probe implantation levels was not significantly different.

4.2. Bioequivalence/bioavailability studies

In spite of the many successful reports of bioequivalence/bioavailability studies in rats, only two human studies have yet been reported [1,12]. The

first compared dermal absorption rate and lag time of lidocaine from a microemulsion vehicle and a commercially available o/w emulsion (Xylocain 5%) in eight subjects, with an experimental design similar to an earlier rat study by the same group [11]. Assessment of pharmacokinetic parameters according to a compartmental model, demonstrated a significant increase in absorption rate and decrease in lag time when lidocaine was applied in a microemulsion compared with an o/w emulsion. Prilocaine was used as relative recovery calibrator, and recovery was monitored during the experiments for each probe via the retrodialysis method. Relative recovery ranged from 56 to 95% during the study, and varied both within and between probes, indicating a benefit of monitoring relative recovery during the experiments.

The second human bioavailability study compared tissue and plasma levels of 8-methoxypsoralen (used in the treatments of many dermatoses together with ultraviolet A exposure) after oral administration (1 mg/kg) and after topical application in either 95% ethanol bath (3 mg/l) or a cream (0.1%, w/w) [1]. Via dermal microdialysis the study elegantly showed that skin/plasma ratio of 8-methoxypsoralen was 3–5 orders of magnitude larger with topical administration compared to oral administration. Thus, the topical route could potentially greatly diminish the severe side-effects observed after oral treatment. The study design also allowed a clear establishment of t_{max} with both administration route where add-on therapy with ultraviolet A exposure should be initiated for maximum therapeutic effect.

Cutaneous levels of a substantial amount of drugs have been determined in humans by the microdialysis technique (in single formulation studies), indicating the tremendous potential for bioequivalence studies, which yet has to be realised.

5. Reproducibility of the microdialysis technique

As indicated by many of the studies described in the review section of animal and human investigations, a relative large variability is often found in cutaneous drug delivery determined by the microdialysis technique (CV typically 50–100%). This may hamper bioavailability studies between formulations that do not differ greatly in cutaneous delivery of a drug and general precision of experiments. An example of the deviation in dermal levels of lidocaine applied in a topical vehicle between subjects and between paired probes in the subjects is illustrated in Fig. 5. The factors which induce this large variability are frequently a subject of debate in the current development and acceptance of the microdialysis technique for pharmacokinetic studies.

The recovery of substances by microdialysis

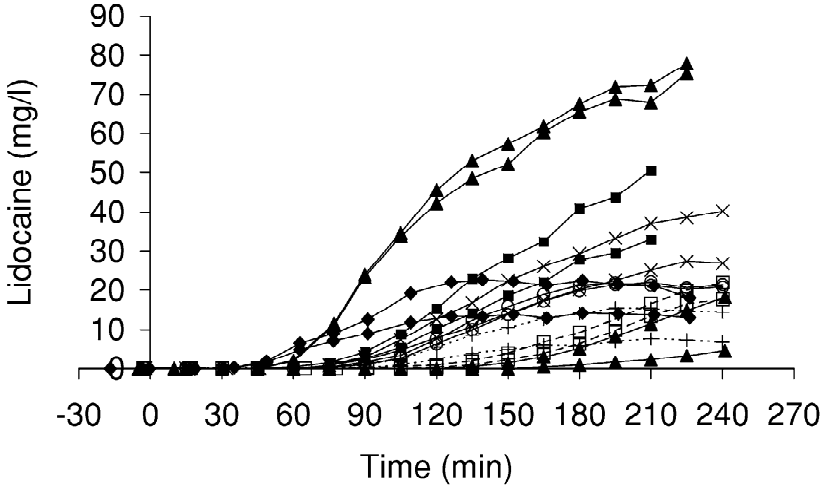

Fig. 5. Inter- and intra-individual variability of lidocaine penetration into the dermis, sampled by microdialysis from eight subjects (two probes under each topical application). The paired probes in each subject are indicated by the same symbols/lines. Raw data from Ref. [12].

probes has, in controlled environments (glass beakers) in vitro, been shown to be highly reproducible (CV typically $<5\%$) independently of probe design and perfusate [3,11,19,55], indicating that the variability is not attributable to the general dialysis sampling technique.

The significance of probe implantation depth on the reproducibility of microdialysis experiments is still a subject of debate in the current literature [3–5,8,9,20,23]. While most authors have not found a correlation between penetrated drug levels in the skin and probe depth [3,4,8,9,20,32], two studies have indicated a correlation [5,23]. Müller et al. [23] found a correlation between absolute recovery of nicotine in humans from a transdermal patch and probe positions ranging from 2 to 9 mm beneath the skin surface (estimated by AUC, $r^2=0.6$; estimated by C_{ss}, $r^2=0.7$). In contrast, Hegemann et al. [3] did not find a correlation between probe depths (ranging from 0.57 to 1.22 mm) and C_{max} of nicotine in the dermis, delivered from a transdermal patch. In a later, experimentally similar study, Müller et al. [8] did not observe a statistical significant correlation between dermal AUCs of a topically applied 'finite' dose of diclofenac and probe depth. A weak correlation ($r^2=0.3$) between dermal delivery of salicylic acid (estimated by AUC) and probe depths in rat skin ranging from 0.5 to 1.1 mm has been indicated [5]. The correlation could, however, not be confirmed in an experimentally similar study in humans [4].

The applied dosage amount, the partitioning and elimination pathway of the current drug are factors which will affect the concentration gradient of the drug in the skin layers, and thereby influence the degree of correlation found in the above-mentioned studies.

Variability of pharmacokinetic parameters from microdialysis experiments generally appears to be larger between individuals compared to within individuals, when multiple probes are used for the assessment of cutaneous penetration from a formulation [5,11,12,32]. In a human study [12], pharmacokinetic profiles of cutaneous lidocaine concentrations tended to be similar for paired microdialysis probes under the same application site). This was reflected in substantially lower mean intra-individual CV (4%) of lag time compared to the interindividual CV (38%). Also mean CV of absorption coefficients was substantially lower intra-individually (30%), in comparison to interindividually (46%). A study by Stagni et al. [20] indicated that day-to-day intrasubject variability ($n=3$) of iontophoretically delivered propranolol in terms of AUC was not significantly different from intersubject variability ($n=11$). The authors concluded that AUC variability was more dependent on the condition of the skin at the time of the experiment, than genetic differences among subject. The correlation between propranolol AUC and various subject demographics was also examined, but neither sex, race, age nor weight could account for the variability.

In vitro/in vivo correlation of the variability of dermal steady-state levels of topically applied 5-fluorouracil in rat skin, have demonstrated a 10-fold difference in in vivo levels determined by microdialysis sampling, and an almost 5-fold difference in excised rat skin levels in vitro similarly determined by microdialysis sampling in the dermis [16]. The in vitro experiments were carried out in a controlled environment by Franz-type diffusion cells, with a fixed area and direction of the diffusion (0.6 cm^2), and the eliminating environment was limited to passive diffusion into the aqueous receptor phase from the surface area of the dermis, i.e., no active elimination by enzymes of vascular supply. These results suggest that the main variability is therefore attributable to individual differences in skin barrier function. However, the diminished variability of the in vitro experiment indicates that distribution and elimination of the drug are important factors, which also contribute to the variability of penetration studies using the microdialysis technique.

The good reproducibility of the microdialysis technique itself by sampling in the skin (and subcutaneous tissues [56]) is also well illustrated by the generally lower variability of dermal drug levels, following systemic administration (CV typically $<30\%$), which eliminates the variability induced by differences in skin barrier function [56–58]. In these studies variability of pharmacokinetic parameters determined with microdialysis in the skin was not substantially different from variability of parameters determined directly from plasma samples [56,58].

The investigated pharmacokinetic parameter additionally influences the size of the variability. Cutaneous drug levels are dependent on both skin barrier properties (i.e., penetration rate and lag time), volume of distribution, elimination rate and additionally

for the microdialysis technique, factors which affect recovery (described in section). Accumulation of these variables in the assessed pharmacokinetic parameter, e.g., AUC and C_{ss}, will inevitably increase overall variability. Thus, to increase precision in estimation of drug penetration rates, it would be useful to differentiate between these factors and eliminate the additional deviations originating from other variables than the penetration rate. The benefit of this is illustrated by the low variability (CV typically $<25\%$) of the classical Franz-type diffusion cell in vitro studies using a confined receptor and donor chamber to estimate permeation of substances though excised skin, where the major variable in steady-state permeation rate is the skin barrier function (roughly assessed). A significant reduction in variability of assessed cutaneous lidocaine penetration in humans from two different formulations (microemulsion and Xylocain 5%), has been demonstrated by individual assessment of apparent absorption rate, lag time and elimination rate in comparison to AUC [12]. CVs of AUCs ($n=8$) from concentration–time curves of lidocaine penetration from the microemulsion formulation was 91%, and was reduced to, respectively, 62 and 37% by estimation of absorption coefficient and lag time. Similarly for the Xylocain 5% formulation, CV was reduced from 63% (AUC) to, respectively, 30% (absorption coefficient) and 39% (lag time). However, this clear trend could not be demonstrated in a similar microdialysis studies in rats [11], possibly due to the low number of replicates ($n=3$). A significantly lower CV has also been reported for propranolol elimination rate (40%) compared to AUC (130%) in human skin [20].

The variability of cutaneous drug delivery assessments by the microdialysis technique is therefore probably mainly attributable to differences in barrier function of the skin, but also to differences in lag time, elimination rate and possibly volume of distribution. Furthermore, probe implantation depth and relative recovery (elaborated below) may also contribute to the variability.

5.1. Inter- and intra-individual variability of recovery

While individual relative recovery assessment is widely integrated into brain microdialysis [37,40,42,43,59–61], only few microdialysis studies of cutaneous drug delivery have yet included the method to assess variability between individual assessment sites and/or fluctuations within the experiments.

Using the retrodialysis by drug method, Müller et al. [23] have demonstrated in 15 subjects a substantial variation in individual recovery in (sub-)cutaneously implanted probes (2–9 mm from the skin surface) ranging from 25 to 36% for nicotine and 1–3% for estradiol, and in a later study in 11 subjects, had a relative recovery of $63\pm15\%$ (mean$\pm$standard error [S.E.]) for diclofenac, with probes implanted 3.9 ± 0.3 mm from the skin surface [8].

A large deviation in relative recovery values of lidocaine and prilocaine has also been demonstrated in both rats [11] and humans [12]. Inter-=individual relative calibrator recovery of prilocaine and lidocaine in the dermis of rats varied between 58–98% and 69–91%, respectively. Furthermore, recovery fluctuations were also observed within the experiments for each probe, and for some probes with a slightly decreasing recovery (most prevalent in the initial phase) during the experiment. The average recovery fluctuation within experiments for prilocaine was $11.3\pm4.2\%$ ($n=29$) with a maximum fluctuation of 21.2% during a single experiment, and for lidocaine the average was $6.1\pm3.1\%$ ($n=20$) with a maximum of 11.4%. Similarly in the human microdialysis study, relative recovery varied between 56 and 95% between the experiments, and a slightly decreasing recovery was also occasionally observed during the experiments. The average recovery fluctuation within experiments was $12.4\pm4.7\%$ ($n=31$) with a maximum fluctuation of 23.1% during a single experiment. The demonstrated inconsistent relative recovery within the experiments, correlates well with previous microdialysis reports in alternative tissues and with other drugs, where large fluctuations and up to 50% decrease in relative recovery has been observed during the experiment [16,39,41,43,60,62,63]. This suggests a substantial increase in accuracy of assessing true extracellular tissue concentrations by estimating recovery during the experiments, contrary to the current habit of only assessing one mean recovery value in cutaneous microdialysis studies. However, not all drugs and tissues display time-dependent recovery

[40,59,60,64]. Thus, the fluctuations in relative recovery of a substance during the experiment appear to be dependent on the diffusion/distribution properties of the substance, the tissue and the time interval studied.

While the mechanism behind the time-dependent fluctuation in relative recovery observed in several studies has not yet been elucidated, several hypothesis on the subject have been published. Larsson [39] suggested that the observed large continuous decrease in recovery of caffeine in the jugular veins and lungs of rats over a 6-h period, was attributable to a deteriorating systemic circulation during narcosis, which decreased diffusion (clearance) of the drug in the tissues/blood in the vicinity of the probe. Sauernheimer et al. [62], suggested that a time-dependent 50% decrease in relative recovery of acetaminophen in the jugular vein of freely moving rats over 15 h, may be due to an accumulation of free cells and macromolecules in the blood on the microdialysis membrane, hindering drug diffusion. Also a rapid decline of recovery in the brain was observed (up to 1 h after probe implantation), followed by a steady-state level. The hypothesis that the actual probe recovery efficiency may change over time, e.g., due to clotting of the fibre membrane, has been supported in a study by Wang et al. [43]. By validating the relative recovery of microdialysis probes (CMA/10) in vitro before and after an in vivo experiment in the ventricle and brain of rabbits, a decrease in relative recovery during a 6-h in vivo experiment was demonstrated to be attributed to a decrease in actual probe recovery efficiency. In contrast to the above-mentioned hypothesises, Lönroth and collaborators [41,63] have suggested that the frequently observed declining recovery assessed by the retrodialysis method, does not reflect actual changes in relative recovery, but is due to an error of the assessment method. The retrodialysis by calibrator method estimates loss of the calibrator from the perfusate, which is assumed = to reflect recovery of a similar compound of interest. A prerequisite for this method is sink conditions in the vicinity of the probe. A decrease in relative recovery of subcutaneous glucose in rats over a 3-h period, indicated by simultaneous retrodialysis with radiolabelled glucose, was presumed to be attributable to an accumulation of the calibrator around the probe, hampering sink conditions [63]. These observations were not evident in the human experiments of the study, however. The hypothesis may be justified for this specific study, considering that the probes were implanted in the subcutaneous fatty tissues, which may have impeded the outwards diffusion of the extremely hydrophilic model drug. However, this hypothesis is not a likely explanation of the time-dependent decrease in recovery observed, for example, in microdialysis studies in the blood vessels, where the vicinity of the probe is continuously perfused.

A decrease in lidocaine/prilocaine relative recovery was observed in both rats [11] and humans [12] during the first 60–90 min of the majority of experiments. Lidocaine is substantially influenced by capillary blood flow, and topical application of lidocaine on rat skin, stripped for stratum corneum, is rapidly cleared from the dermis [65]. It is therefore likely that the observed slight decrease and fluctuation of relative recovery over time in these studies, may be attributed to variations in blood flow. It is generally acknowledged that implantation of microdialysis probes are associated with a temporary increase in cutaneous blood perfusion, skin thickness and histamine release [29,30,41], which slowly subsides during the following hours. Additionally, minor local bleeding from disruption of the capillaries by implantation has been observed [41]. All these factors influence the environment of the probe, which can affect diffusion and clearance of the substance i.e., relative recovery. Lidocaine is a weak vasoconstrictor, which could also have attributed to decreased clearance. Also a significant correlation ($r^2 = 0.51$, $P < 0.002$, $n = 16$) has been demonstrated between mean relative recovery and elimination rate of lidocaine in humans [12], supporting the influence of vascular clearance.

A drawback concerning the reproducibility of the retrodialysis method to estimate relative recovery and the linear probe design used for the majority of cutaneous drug delivery studies today, is that variations in active fibre length outside the actual application area (when the membrane ‘window’ has not been confined to the application area), will influence loss of the calibrator in a reverse proportional direction to the actual recovery of the drug, and lead to an overestimation of relative recovery. Further-

more, analytical errors will also result in inverse proportional errors in estimation of relative recovery, which will be particularly sensitive for the correction of dialysate sample concentrations if relative recovery is very low and lead to large fluctuation of corrected concentration–time curves [40].

5.2. Pharmacokinetic compartmental models

The main parameters of interest in topical bioequivalence/bioavailability studies are the duration of time before the substance enters the skin from time of administration (i.e., lag time), and the speed of which the substance is absorbed into the skin (i.e., penetration rate). Application of an appropriate compartmental pharmacokinetic model to fit concentration–time curves, will enable estimation of these parameters. A further advantage of introducing a compartmental pharmacokinetic model to analyse microdialysis data, is the possibility of individual estimation of the pharmacokinetic parameters, which can lead to a lower variability of the results as described above.

A relatively simple compartmental model has been introduced for analysis of cutaneous microdialysis data from topically applied drugs (lidocaine and prilocaine hydrochloride) in both rats [11] and humans [12] according to a zero-order absorption (R_0), one compartment, and first-order elimination (k) model, including a lag time (t_{lag}):

$$C = \frac{K_{abs}}{k}\left(1 - e^{-k(t - t_{lag})}\right) \quad (4)$$

where K_{abs} is the absorption coefficient. The absorption coefficient K_{abs} estimates the initial rate of concentration change (at time $= t_{lag}$). Mean correlation between observed and predicted drug concentrations was 0.981 ± 0.029 ($n = 49$) for the rat study and 0.994 ± 0.007 ($n = 29$) for the human study (example of average fit: Fig. 6). The current study design did not allow for individual estimation of absorption rate and volume of distribution (V_d), and apparent absorption rate was, therefore, estimated as an absorption coefficient ($K_{abs} = R_0/V_d$). It was assumed that V_d was similar (within the limitations of general biological variability) for a drug applied in the same anatomical region and species and that the absorption coefficient, therefore, was a reliable indicator of apparent absorption rate.

In a microdialysis study of transdermal drug delivery, although not performed in the subcutaneous layer, Müller et al. [8] used two standard compartmental pharmacokinetic models to assess C_{max} and AUC of topically applied diclofenac in two defined tissue layers, 4 and 9 mm, respectively, below the skin surface. The concentration–time curves from the upper and lower layer, were fitted to a two-

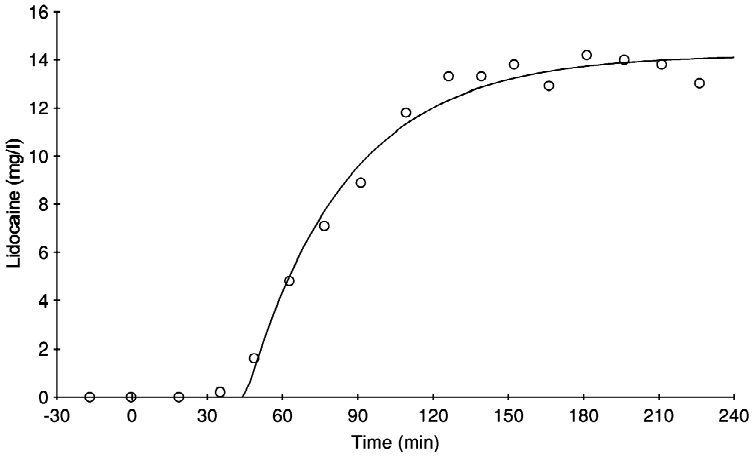

Fig. 6. Typical pharmacokinetic model fit to cutaneous microdialysis concentration–time curves. Open circles represent actual dialysate concentrations (corrected for recovery) and solid line represents predicted concentrations based on the pharmacokinetic model [12].

compartment model and a one-compartment model, respectively, with a first-order elimination and absorption rate, without lag time. However, the compartmental parameters were not reported.

6. Correlation between in vivo microdialysis and alternative methods to assess cutaneous drug delivery

The microdialysis technique uniquely enables assessment of drug levels directly in the dermis and appears to be a very sensitive method to investigate minor differences in cutaneous drug delivery, which has not been possible with previous methods. However, as the technique has only recently been introduced to cutaneous drug delivery studies, it is naturally of great interest to evaluate the method and compare it to well-established in vitro/in vivo models and readdress the clinical relevance of the 'well established' methods.

6.1. In vitro models

One of the most acknowledged methods for bioequivalence studies in vitro is the Franz-type diffusion cell [2,66–68], which has been used for decades to assess qualitative permeation ranks of topical formulations. A very close relationship between permeated 5-flouruoracil levels in the receptor compartment of a Franz-type diffusion cell, sampled by regular sample withdrawal and the levels (corrected for relative recovery) assessed by microdialysis sampling in the same compartment has been demonstrated [47]. A good qualitative agreement between flux through different rat skin samples ($n=3$) determined from traditional receptor compartment sampling, and dermal levels assessed by the microdialysis technique in an in vitro diffusion cell, has furthermore been demonstrated for the drug [16]. However, comparison of average dermal drug levels sampled by microdialysis from rat skin in vitro and in vivo [16], has indicated that 5-fluorouracil levels assessed in vitro are approximately 40 times higher than the actual in vivo levels; hence suggesting that quantitative assessments based on in vitro experiments are not reliable indicators of in vivo levels due to the intact clearance processes (vascular and enzymatic) in the latter.

A good qualitative and relative quantitative correlation has been demonstrated between in vitro permeation using Franz-type diffusion cells [69] and in vivo dermal absorption coefficient determined by microdialysis [11] of a lipophilic (lidocaine) and hydrophilic model drugs (prilocaine hydrochloride/lidocaine hydrochloride), respectively, applied in four different topical vehicles. A linear in vitro/in vivo relationship of cutaneous absorption from the vehicles was indicated for the lipophilic model drug ($r^2=0.97$) and the hydrophilic model drugs ($r^2=0.86$), respectively. However, the in vitro/in vivo assessments did not correlate between the model drugs. In vitro flux of the formulations containing the hydrophilic model drugs, indicated that cutaneous delivery rate of the drugs would on average be 20% relative to the mean delivery rate of the lipophilic model drug from the four vehicles. With the microdialysis technique though, it was demonstrated that the mean dermal delivery rate in vivo of the hydrophilic model drugs was only approximately 2% relative to that of the lipophilic drug. These studies suggest that in vitro Franz-type diffusion cells may be a good qualitative, and relative quantitative indicator of bioequivalence between formulations with the same drug; however, this method is not reliable for assessment of relative drug delivery rate between different drugs, and does not enable estimation of actual dermal drug levels in vivo. Microdialysis has also been used to demonstrate significant increase of cutaneous diclofenac [14] and enaxin [13] delivery in rats by application of various enhancers, which correlated qualitatively well with transdermal permeation in vitro in the former, but not as clearly in the latter study.

6.2. In vivo models

To the author's knowledge, no comparison study of the microdialysis technique and the previously most prevalent assessment technique for direct estimation of cutaneous penetration in vivo, tape stripping, has yet been performed.

Two comparison studies by Murakami et al. [6,7] of cutaneous drug delivery in rats by the microdialysis technique and by the indirect assessment of plasma concentrations have been published with diverging findings. One report [7] demonstrated an excellent quantitative correlation between dermal

AUC/C_{max} of topically applied tranilast in six different vehicles, assessed by the microdialysis technique, and AUC/C_{max} from plasma concentration–time curves. The other report [6], however, did not find any correlation between dermal AUC/C_{max} and plasma AUC/C_{max} of salicylic acid applied in five different vehicles. The studies illustrate that plasma levels do not always correlate with dermal drug delivery rate, and furthermore do not provide information of actual drug levels in the skin.

Among the conventional in vivo methods to assess cutaneous drug delivery is, additionally, pharmacodynamic models. A combination of the microdialysis technique and a pharmacodynamic model is an attractive solution to assess PK/PD relationships, as the sampling can be performed in the target organ. The cutaneous absorption of lidocaine has been investigated in humans by the microdialysis technique and compared to the anaesthetic effect of a microemulsion formulation and Xylocain 5% [12]. The pharmacodynamic study showed a significant reduction in pain perception of prods with von Frey hairs during the application of the two lidocaine formulations, discernible from placebo treatment. The anaesthetic effect of the two formulations was not statistically discernible, although indicating similar cutaneous absorption profile of lidocaine from the two formulations. However, the cutaneous microdialysis study demonstrated a 3-fold increase in apparent absorption rate and a significant decrease in lag time of lidocaine applied in the microemulsion vehicle compared to Xylocain 5%, which resulted in a more than four times increase in total amount of lidocaine absorbed into the skin from the former vehicle. No correlation between the pharmacokinetic parameters and AUE could be demonstrated. It was suggested that the efficacy of lidocaine in the assessed concentration range was relatively low, which hampered differentiation between the formulations in the pharmacodynamic study. Regardless, the study illustrated the higher sensitivity and lower variability of the microdialysis technique to assess bioequivalence of cutaneous drug delivery, compared to the pharmacodynamic model.

7. Prediction of cutaneous drug delivery in humans from rat microdialysis studies

No significant difference was found between cutaneous absorption coefficient of lidocaine in Wistar rats (administered on the side of the thorax) [11] and in humans (administered on the volar surface of the forearm) [12] from a microemulsion vehicle and Xylocain 5%, respectively. As the estimated absorption coefficients ($K_{abs} = R_0/V_d$) did not account for differences in V_d between rat and human skin, the actual ratio between absorption rates may differ slightly from the observed absorption coefficients. Rat skin is generally thinner than human skin, both in regard to whole skin and stratum corneum thickness (10–15 μm in rat skin compared to 15–20 μm in human skin), and contains fewer cornified cell layers. It may therefore be assumed that the volume of distribution is lower in rat skin, compared to human skin, and that the assessed penetration rates in rats, are slightly overestimated, relative to those in humans. This would be in accordance with the general findings of other assessment techniques, where rat skin is typically observed to be two to five times more permeable than human skin [68]. Also, elimination rate of lidocaine in human and rat skin was not discernible (unpublished data from Refs. [11,12]). However, lag time for lidocaine detection in the dermis was increased approximately 5.5-fold for both the microemulsion formulation and Xylocain 5%, which corresponds well with the increased thickness and additional cell layers of the human stratum corneum. This indicates that the total time for lidocaine passage through the barrier layer of the skin increased more than the rate of the passage.

Comparative studies of cutaneous drug penetration in hairless rats and the volar forearm of humans, using the microdialysis technique, have also been done by Benfeldt et al. [4,5,70]. These studies indicated a 53-fold increase in dermal penetration (assessed by $AUC_{0-210\ min}$) of topically applied salicylic acid for rats relative to humans (applied on the volar surface of the forearm). These findings are in contrast to an in vitro study, which demonstrated similar permeation rates of salicylic acid through excised skin from hairless rats and human breast and thigh [71]. While in vitro studies are not always reliable indicators for the in vivo situation, the very large differences between the findings of these studies suggest that penetration rate alone cannot explain the differences in AUC found by Benfeldt et al., and that deviating elimination rate, lag time and

possibly volume of distribution may have contributed to the observed differences. The different anatomical region of human skin of the two studies, may also have contributed to the deviations. The in vivo studies did, however, indicate a good relative rat/human correlation between AUC ratios of penetration of salicylic acid through normal skin and various degrees of perturbed skin, indicating that the rat model was a good indicator for relative decrease in human skin barrier function to drug penetration in different stages of perturbation. While both the formulation and microdialysis probes were different in the studies, hampering quantitative correlations, diclofenac has been shown to penetrate the skin very slowly when no enhancers are applied in both rats [14] and humans [8].

Human/rat microdialysis correlation studies, have indicated that relative recovery does not differ substantially between the two species [4,5,11,12]. Benfeldt et al. found an average relative recovery of salicylic acid in rats of 29±4% and in humans of 24±4%. The average relative recovery of prilocaine has been shown to be 81±6% in rats [11] and 77±7% in humans [12]. While in vitro recovery is often not a reliable indicator of relative recovery in vivo [4,5,23], it appears that recovery in human skin may be derived from rat experiments; however, more studies are desirable to establish the relationship more closely.

The most important determinant for the rat–human correlation is most likely the properties of the model drug, which will be affected in various degrees by the differences in stratum corneum structure and lipid composition between rat and human skin. However, the multiple biological factors (i.e., anatomical location of the administration), which influence the permeability of the skin, and the rat species/age [72], are most likely parameters that additionally contribute to the observed differences in the various studies.

8. Conclusions

The microdialysis technique is substantially more sensitive and less invasive than previous in vitro/in vivo methods in assessing differences in cutaneous penetration rates of substances and barrier function of the skin. It has been demonstrated to enable quantification of numerous hydrophilic and a few moderately lipophilic drugs directly in the target organ—the viable layers of the skin. Studies in both humans and rats have shown a tremendous potential of microdialysis to estimate bioequivalence/bioavailability of topical formulations, due to the high sensitivity of the technique. However, a considerable variability of dermal drug levels by microdialysis sampling of cutaneously absorbed drugs has been observed in the early studies, which hampered the precision of bioequivalence studies. The main variability is indicated to be attributable to interindividual differences in barrier function of the skin; however, differences in lag time, elimination rate and possibly distribution also contribute significantly to the variance between assessment sites. It has been shown that a substantial increase in precision of cutaneous drug delivery assessment can be obtained by estimation of absorption rate, lag time and elimination rate through compartmental modelling of microdialysis data. Furthermore, a few studies have indicated a lower variability of pharmacokinetic parameters when assessment sites are corrected individually for relative recovery. Probe depth appears mainly to influence reproducibility of the technique, when implantation ranges between larger distances than the cutaneous layers.

Assessment of bioequivalence in terms of absorption rate by in vivo microdialysis appears to correlate qualitatively well with established in vitro Franz-type diffusion cell assessments of permeation rates for formulations with the same drug. However, there does not appear to be a correlation between cutaneous in vitro flux and in vivo absorption for formulations with different drugs, and in vitro methods are not reliable indicators of actual tissue drug levels in vivo. Furthermore, assessment of systemic levels has been demonstrated to not always adequately estimate relative dermal absorption rates, and in vivo microdialysis is currently the only technique to directly assess unbound drug levels in the dermis of the skin.

Finally, a good correlation between in vivo cutaneous absorption coefficient and elimination rate of a lipophilic model drug in Wistar rats and in human forearm skin has been indicated by a single study. However, lag time appears to be much longer in

human skin than in rat skin, emphasising the importance of differentiating between these parameters, to enable quantitative prediction of bioequivalence for topical formulations in humans from rat microdialysis studies. More studies are needed to confirm these relationships.

The future challenges for assessment of cutaneous drug delivery using the microdialysis technique lies in the sampling of lipophilic compounds, which constitutes the majority of novel drugs targeted to the skin. Thus, further work is needed in the optimisation of perfusate, diminishing adhesion to microdialysis materials and also to implement more sensitive analytical techniques. But as the many authors cited in this review have clearly demonstrated, the technique can already now be applied for biopharmaceutical optimisation of topical formulations with the numerous successfully recovered drugs.

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