Dermal Pharmacokinetics of Microemulsion Formulations Determined by *In Vivo* Microdialysis

Mads Kreilgaard[1,2]

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Purpose. To investigate the potential of improving dermal drug delivery of hydrophilic and lipophilic substances by formulation in microemulsion vehicles and to establish a reliable pharmacokinetic model to analyze cutaneous microdialysis data.

Methods. After a topical application of microemulsions, commercially available creams, and a hydrogel, unbound cutaneous concentrations of lidocaine and prilocaine were determined by *in vivo* microdialysis in rats. Recovery was monitored during the experiments via retrodialysis by calibrator.

Results. The presented pharmacokinetic model provided an excellent fit of the microdialysis concentration–time curves with reliable estimation of absorption coefficient and lag time. The microemulsion formulations were shown to increase the absorption coefficient of lidocaine more than eight times (753 μg/l/min) compared with a conventional oil-in-water emulsion-based cream (89 μg/l/min) and prilocaine hydrochloride almost two times (8.9 μg/l/min) compared with hydrogel (5.2 μg/l/min).

Conclusions. The microemulsion formulations can be applied to increase dermal drug delivery of both the hydrophilic and lipophilic model drug. The pharmacokinetic model presented in this report is, to the author's knowledge, the first example in the literature, providing reliable estimation of cutaneous absorption coefficient and lag time from microdialysis data of topically applied substances.

KEY WORDS: microemulsion; microdialysis; pharmacokinetic; dermal; lidocaine; prilocaine hydrochloride.

INTRODUCTION

In the effort of exploring the potential advantages of transdermal/dermal drug delivery (e.g., minimal first-pass metabolism, patient comfort/compliance, local drug delivery to the skin) various physical and chemical approaches have been used to overcome the limiting barrier of drug penetration into the skin. Several reports have indicated that microemulsion vehicles have a high transdermal delivery potential for both lipophilic and hydrophilic drugs compared with conventional vehicles (1–8). However, the cutaneous drug delivery potential of microemulsions has been observed not only to be dependent on the applied constituents of the vehicle but also drastically on the composition/internal structure of the phases, which may hamper drug diffusion in the vehicles (1,9,10). The multiple favorable reports of cutaneous drug delivery potential of microemulsions, have, however, mainly been performed *in vitro.* Only few reports have confirmed this potential *in vivo* (4,11)—presumably because of the novelty of the vehicles for topical administration and to the lack of appropriate *in vivo* techniques to assess skin absorption.

Recently, the microdialysis technique has been introduced to dermatological research (12) as a valuable *in vivo* tool that is minimally invasive and enables assessment of full local pharmacokinetic profiles of cutaneous drug penetration from each sampling site (13,14). However, a relative large variation in individual dermal drug levels found, not only between individuals, but also frequently between microdialysis probes, has hampered bioequivalence studies (14). Beside the biological variation in stratum corneum barrier function, which generally leads to a considerable variation in skin absorption studies, other variables are introduced by the microdialysis technique, e.g., intra- and interindividual alterations in relative recovery of the substance of interest. Recovery of the substance is affected by clearance of the drug from the tissues surrounding the microdialysis membrane, i.e., diffusivity of the substance and capillary blood flow around the probe and the partition coefficient between the tissues and the perfusate. These parameters may possibly be subject to changes both during an experiment and between assessment sites. To monitor recovery during the experiment and between assessment sites, retrodialysis by calibrator have successfully been introduced to microdialysis in lung/blood (15) and brain (16–18) of rats. However, an individual relative recovery assessment has not yet been implemented as a standard procedure to microdialysis studies of cutaneous drug delivery. Furthermore, bioequivalence studies with this novel technique have been hampered by the lack of an appropriate pharmacokinetic model to individually assess absorption rate, lag time and elimination rate from cutaneous microdialysis data.

The objective of this study was to evaluate *in vivo* dermal drug delivery potential of microemulsions with alternating compositions for a lipophilic and a hydrophilic model drug, compared to a commercially available oil-in-water (O/W) emulsion, hydrogel, and eutectic mixture-based formulation using the microdialysis technique. Additionally, the aim of the study was to introduce recovery monitoring during the study by the retrodialysis by calibrator method, to obtain more reliable estimates of true unbound drug tissue concentrations. Furthermore, a compartmental pharmacokinetic model to analyse cutaneous microdialysis data is presented, and *in vitro* results from Franz diffusion cells using rat skin and *in vivo* rat microdialysis are compared.

MATERIALS AND METHODS

Chemicals

Labrasol® (Caprylocaproyl Macrogolglycerides), Plurol Isostearique® (polyglyceryl isostearate), and isostearylic isostearate (>92% purity) (Gattefossé, Lyon, France) were donated from Bionord A/S (Hellerup, Denmark). The same batch of the microemulsion components was used throughout all experiments. Lidocaine and prilocaine hydrochloride were

[1] The Royal Danish School of Pharmacy, Department of Pharmaceutics, Copenhagen, Denmark.

[2] To whom correspondence should be addressed at H. Lundbeck A/S, Neurochemistry & Discovery ADME (845), Ottiliavej 9, DK-2500 Valby, Denmark. (e-mail: makr@lundbeck.com)

ABBREVIATIONS: C_{in}, drug concentration in the perfusate; C_m, drug concentration in the medium; C_{out}, drug concentration in the dialysate; HPLC, high-performance liquid chromatography; J, flux; k_e, elimination rate; k_{abs}, absorption coefficient; L, lidocaine; O/W, oil-in-water; P, prilocaine hydrochloride; RR, recovery; R_0, penetration rate; t_{lag}, lag time; V_d, volume of distribution

purchased from Unikem (Copenhagen, Denmark) and Sigma Chemicals (St Louis, MO), respectively. All chemicals were used as received. Hypnorm® (0.315 mg/ml Fentanyl and 10 mg/ml Fluanison) was obtained from Janssen-Cilag (Beerse, Belgium) and Dormicum® (5 mg/ml midazolam) from Roche (Basel, Switzerland). EMLA® (2.5% lidocaine, 2.5% prilocaine), Xylocain® 5% cream (lidocaine), and Xylocain® 2% gel (lidocaine hydrochloride) (Astra, Södertälje, Sweden), are commercial products. Distilled water filtered through a Milli-Q filter (Millipore Corporation, Bedford, MA) was used throughout the experiments. Solvents were of high-performance liquid chromatography (HPLC) grade, and all other chemicals were of analytical grade.

Formulation of Microemulsions

Microemulsion compositions (referred to as system A, D, E and G) (Table I) and drug concentrations were chosen from pre-constructed pseudo-ternary phase diagrams and characterisation studies (1). The microemulsion formulations were spontaneously formed at room temperature by admixing appropriate quantities of the compounds.

Microdialysis System

The microdialysis system consisted of a CMA/100 microinjection pump (CMA/Microdialysis AB, Stockholm, Sweden) equipped with 2.5-ml Exmire microsyringes (ITO Corporation, Fuji, Japan). An isotonic Glucose-Ringer solution (Na^+ 118.3 mM, PO_4^{3-} 2 mM, Mg^{2+} 1.2 mM, Ca^{2+} 1.2 mM, K^+ 5.0 mM, Cl^- 124.5 mM, D(+)-Glucose 2.0 mM) (note: Na^+ 165.4 mM, PO_4^{3-} 47.2 mM, Cl^- 110.2 mM were used for the experiments with G 7.5% L) buffered at pH 6.5 was used as perfusate at a flow rate of 1.2 μl/min during all experiments. Lidocaine or prilocaine (30 mg/l) was added to the perfusate as recovery calibrator for the *in vivo* penetration studies of prilocaine and lidocaine formulations, respectively. No calibrator was used for the penetration studies with EMLA.

Custom-made microdialysis probes with a linear design (19), based on single dialysis fibre (208-μm inner diameter (ID), 216-μm outer diameter (OD), 2 kDa molecular weight cut off) from a dialysator (Gambro GFS +12, Gambro Dialysaten, Hechingen, Germany), were used in this study. After implantation, the fiber was at each end connected to a polythene tube (0.4-mm ID, 0.8 mm OD; Portex, Berck-sur-Mer, France), leading to the microinjection pump and the sampling vial, respectively. Active fiber length was 30 mm and 20 mm for the *in vivo* and *in vitro* experiments, respectively.

In Vitro Microdialysis Validation

Three probes were placed in a 50-ml beaker, filled with a glucose-Ringer solution containing the appropriate drug concentration, and magnetically stirred at 350 rpm at room temperature (25 °C). After an equilibration period of 30 min, dialysate was collected for 20 min and analyzed for lidocaine and prilocaine content by HPLC.

For the regular recovery studies, the surrounding medium contained respectively lidocaine or prilocaine concentrations of 2, 10, 20, 30, or 50 mg/l, and the perfusate contained 30 mg/l of the opposite drug as a control. Relative recovery (RR) was calculated as slope of the linear regression of drug concentration in the dialysate (C_{out}) as a function of drug concentration in the medium (C_m):

$$RR = \left(\frac{C_{out}}{C_m}\right) \quad (1)$$

For the retrodialysis studies, a blank glucose-Ringer solution was filled in the beaker, and the probes perfused with a glucose-Ringer solution containing respectively lidocaine or prilocaine concentration of 2, 10, 20, 30, or 50 mg/l. Recovery at each concentration was calculated as:

$$RR = \left(\frac{C_{in} - C_{out}}{C_{in}}\right) \quad (2)$$

where C_{in} is drug concentration in the perfusate.

In Vivo Microdialysis Studies

Male Wistar rats (280–420 g) were used for the penetration studies. The research adhered to the "Principles of Laboratory Animal Care" (NIH publication #85-23, revised 1985). The animals were anesthetised before the study with a subcutaneous bolus injection of 0.2 ml/100 g body weight of a 1:1:2 mixture of Hypnorm®:Dormicum®:H_2O and maintained in anaesthesia throughout the entire study with supplementary bolus injections of half the initial dose.

Levothoracally the fur was removed with an electric animal hair clipper and the rat placed on a temperature-controller pad (CMA/150, CMA/Microdialysis AB, Stockholm, Sweden) heated to 37.5 °C with the right side of the body facing the pad. Two 22-gauge cannulas were implanted,

Table I. Microemulsion Compositions and Drug Contents

System	Water (% w/w)[a]	Isostearylic isostearate (% w/w)[a]	Percent Labrasol/ Plurol Isostearique (% w/w)[a]	Lidocaine (% w/w)[b]	Prilocaine hydrochloride (% w/w)[b,c]
A	20	10	70[d]	23	5
D	7	70	23[d]	17	–
E	11	26	63[e]	–	2.4
G	65	3	32[f]	9.1/7.5	14

[a] Values are % w/w of initial microemulsion batch without drug.
[b] Values are % w/w drug content of loaded microemulsion.
[c] Values are based on molecular weight of prilocaine free base.
[d] 1:1 mixture.
[e] 2:1 mixture.
[f] 3:1 mixture.

5 mm apart, at the shaven skin site in the dermis, at a length of 30 mm, and resurfacing through exit punctures. Through the tip of each cannula, a microdialysis probe was inserted and the needle retracted, leaving the probe fiber implanted in the skin. Subsequently, the outlet tube was glued to the efferent fiber end, the inlet tube of the probe connected to the microinjection pump with a tubing adapter (CMA/Microdialysis, Solna, Sweden), and perfusion initialized. At the center of the implanted microdialysis fibers, a cylindric polyethylene application chamber (22 mm ID, 3 ml volume) was glued to the skin with cyanoacrylate glue. The rat was allowed a minimum recovery period of 45 minutes after probe implantation to diminish skin trauma (20) before onset of the experiment. After a 15-minute baseline sampling of dialysate, 2 ml of the current formulation was injected into the application chamber through a circular 1-cm hole at the top of the chamber. Dialysis sampling was continued for 3 hours, replacing collection vials every 13 min, and the samples immediately assayed for lidocaine and prilocaine content by HPLC after the experiment. The rat was subsequently sacrificed. Three independent experiments were performed for each formulation. Time–points were calculated as the mid-point between sampling intervals and corrected for lag time of the perfusate from the microdialysis site to the probe outlet. Unless otherwise stated, analyzed dialysate concentrations are corrected for estimated recovery at each sampling interval.

Similar to the *in vitro* investigations, validation of *in vivo* recovery was performed with three probes in one rat using the retrodialysis method, with lidocaine and prilocaine concentrations of 2, 10, 20, 30, and 50 mg/l, collecting dialysate for 20 min after a 30-min equilibration period.

HPLC Assay

Lidocaine and prilocaine were quantified using an HPLC system (Merck-Hitachi, Darmstadt, Germany), consisting of a D-7200 autosampler (7 μl loop), 655A-11 pump operating at 0.4 ml/min, 655A UV-detector operating at 205 nm, and a D-7500 integrator. Analytes were separated by Waters Symmetry™ C-18 column (5 μm, 150 × 2.1 mm) (Milford, MA) and maintained at 35 °C. The mobile phase consisted of acetonitrile/0.05 M aqueous Na_2HPO_4/triethylamine (40/60/0.01, v/v) adjusted to pH 7. The peak area correlated linearly with lidocaine (r^2 = 0.999) and prilocaine (r^2 = 1.000) concentrations in the range 1-60 mg/l. Limit of quantitation was 0.072 mg/l and 0.053 mg/l, coefficient of variation (CV) was 0.7% and 1.1% at 1 mg/l and 3.7% and 2.2% at 50 mg/l for lidocaine and prilocaine, respectively.

Pharmacokinetic and Statistical Analysis

Pharmacokinetic analysis was performed by PC-compatible software WinNonlin™ © ver. 2.1 (Pharsight Corporation, Mountain View, CA). Concentration-time curves of the drugs were fitted to a zero order absorption (R_0), one compartment, and first order elimination (k_e) model, including a lag time (t_{lag}), according to:

$$C = \frac{R_0}{k_e \cdot V_d}\left(1 - e^{-k_e(t - t_{lag})}\right) \quad (3)$$

where V_d is volume of distribution. Because the obtained data did not allow an individual estimation of V_d and R_0, V_d was assumed to remain constant during each study and apparent drug absorption rate estimated as an absorption coefficient ($k_{abs} = R_0/V_d$). Estimation of the parameters k, t_{lag}, and k_{abs} were performed using the Nelder-Mead algorithm minimisation method. The goodness of fit was estimated as the squared correlation (r_y^2) between observed (y) and predicted ($\hat{y}$) drug concentrations according to:

$$r_y^2 = \frac{\sum(yi - \bar{y})(\hat{y}i - \bar{\hat{y}})}{\sqrt{\sum(yi - \bar{y})^2} \cdot \sqrt{\sum(\hat{y}i - \bar{\hat{y}})^2}} \quad (4)$$

Because no calibrator was used for the EMLA studies, concentrations–time curves of lidocaine formulations were analyzed both before and after correction of dialysate concentration by recovery estimates, to enable comparison.

k_{abs} and t_{lag} values were analysed statistically by a two-tailed *t* test. As standard deviation (SD) of k_{abs} values increased proportionally with mean, indicating log-normal distributed data, logarithmic transformation of the values was performed prior to statistical analysis. A $P < 0.05$ was considered statistically significant. All values are calculated from three independent experiments, using the average values of the two probes, and data are expressed as mean ± SD unless otherwise stated.

RESULTS

In Vitro/Vivo Validation

Both *in vitro* and *in vivo,* a linear correlation between medium/perfusate concentrations and recovered dialysate concentration was observed for both lidocaine and prilocaine recovery studies in the range of at least 2–50 mg/l. *In vitro* relative recovery was (Equation 1; mean ± standard error) 74 ± 0.9% (r^2 = 1.000) for lidocaine and 78 ± 3.6% for prilocaine (r^2 = 0.994). *In vitro* recovery, determined by retrodialysis (Eq. 2; mean ± standard error), was 81 ± 1.5% (r^2 = 0.999) for lidocaine and 80 ± 1.5% (r^2 = 0.999) for prilocaine.

In vivo recovery validation, determined by the retrodialysis method (n = 1), confirmed the concentration-independent relative recovery for lidocaine (78 ± 0.5%; r^2 = 1.000) and prilocaine (75 ± 1.9%; r^2 = 0.998). Recovery values were not affected by the altered perfusate composition used for the G 7.5% L experiments (average: 74 ± 7.5%).

In Vivo Microdialysis

Generally, pharmacokinetic profiles tended to be similar for paired probes under the same application site (exemplified in Fig. 1), with largest variation found between rats, indicating a reproducible implantation technique. The expected probe placement in the dermis layer of the skin was visually confirmed on several occasions (approximately 1 mm from the skin surface), by excising the skin of the microdialysis site after the experiment.

Relative calibrator recovery of prilocaine and lidocaine varied between 58–98% and 69–91%, respectively, between experiments. Furthermore, recovery fluctuations were also observed within the experiments for each probe, occasionally with a slightly decreasing recovery during the experiment (Fig. 1). The average recovery fluctuation within experiments

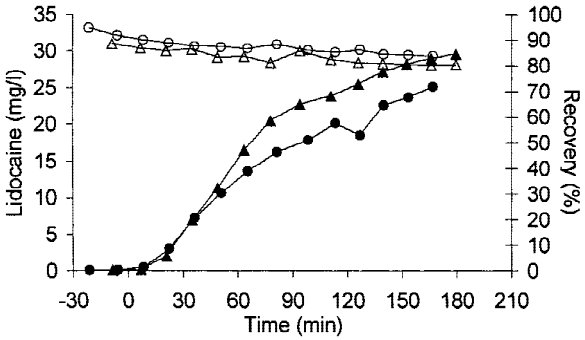

Fig. 1. Example of recovery fluctuation during an *in vivo* microdialysis experiment (formulation G 9% L, rat 3): (▲) lidocaine penetration, probe 1; (△) lidocaine penetration, probe 2; (●) prilocaine recovery, probe 1; (○) prilocaine recovery, probe 2.

for prilocaine was 11.3 ± 4.2% (n = 29) with a maximum fluctuation of 21.2% during a single experiment, and for lidocaine the average was 6.1 ± 3.1% (n = 20) with a maximum of 11.4%.

The proposed pharmacokinetic model (Eq. 3) provided a robust fit for all concentration-time curves. The mean r_y^2 of 0.963 ± 0.056 (n = 49) for data corrected for recovery, was slightly larger than that for uncorrected data (0.958 ± 0.077), indicating slightly less fluctuating data points after recovery correction; however, the values were not statistically discernible. Example of average curve fit is shown in Fig. 2a (r_y^2 = 0.996) and worst-case curve fit in Fig. 2b (r_y^2 = 0.803).

Mean dermal concentration–time curves (corrected for recovery) for lidocaine and prilocaine hydrochloride/lidocaine hydrochloride are illustrated in Fig. 3. Estimation of absorption coefficient and lag time estimates from the individual concentration-time curves, with dialysate concentrations corrected for recovery, are presented in Table II. Lidocaine exhibited larger average absorption coefficients from all three microemulsion compared to a conventional O/W-emulsion based formulation (Xylocain® 5%), with G 9% increasing penetration rate of lidocaine more than eight times compared to Xylocain® 5% (P = 0.004). Lag times were not discernible between the four formulations.

Mean absorption coefficient of prilocaine hydrochloride was approximately doubled when applied in microemulsion G 14%, compared to the hydrogel formulation (Xylocain® 2%), however no statistical significance for this increase could be demonstrated (P = 0.45). Microemulsion A and F did not increase penetration notably. However, estimated average lag time was statistical significantly reduced by administration of prilocaine hydrochloride in microemulsions (A: P = 0.04; E: P = 0.002; G: P = 0.001) compared to the hydrogel.

Absorption coefficient and lag time estimates from concentration–time curves without dialysate recovery correction after application of lidocaine formulations are presented in Table III. EMLA is based on an eutectic mixture of lidocaine and prilocaine, which forms a unique oil phase of the emulsion consisting of the neat local analgesics and is considered the fastest penetrating lidocaine formulation commercially available (1). This creates an extremely high drug activity in the vehicle, which increases the absorption coefficient of lidocaine to about three times larger than Xylocain® 5%. However, microemulsion G 9% still exhibits an almost 3-fold larger absorption coefficient (P = 0.11) compared with

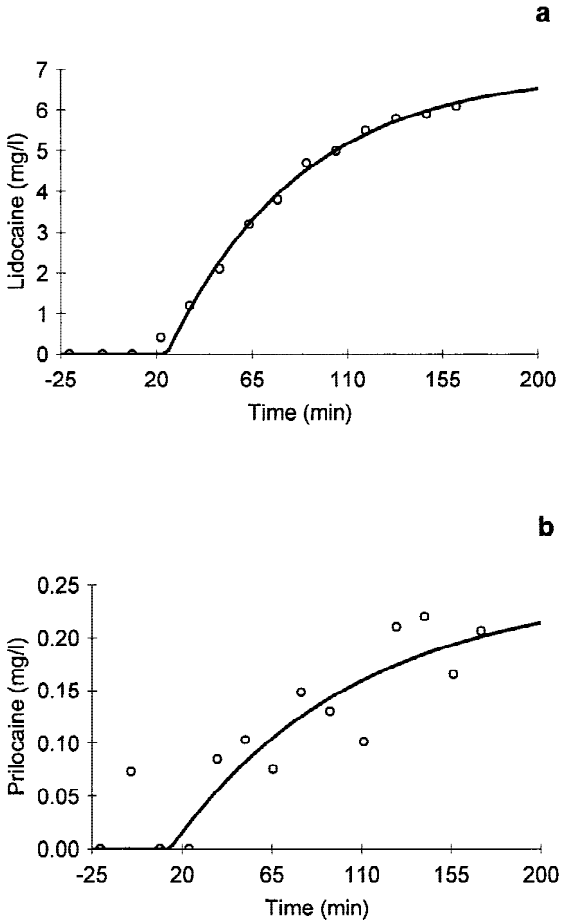

Fig. 2. (a) Typical pharmacokinetic curve fit of concentration-time curves (formulation A 23% L, rat 3, probe 2, r_y^2 = 0.996). (b) Worst-case pharmacokinetic curve fit (formulation E 2.4% P, rat 3, probe 2, r_y^2 = 0.803). Open circles represent actual dialysate concentrations (corrected for recovery) and solid line represents predicted concentrations based on the pharmacokinetic model.

EMLA. Futhermore, average lag time of EMLA is about twice as long compared to those of the microemulsions.

CVs of k_{abs} are generally about twice as large as t_{lag} (Tables II and III), and CVs are not significantly reduced by use of recovery correction.

Linear regression of estimated cutaneous elimination rate (k_e) of lidocaine and average recovery of calibrator during the study, did not indicate a correlation (r^2 = 0.04, n = 29) between relative recovery and elimination rate of the drug from the tissues surrounding the microdialysis probe in the present study.

A linear correlation was found between mean *in vivo* absorption coefficient, and *in vitro* steady-state flux (J) through excised rat skin in Franz-type diffusion cells (1) for both the lidocaine free base (r^2 = 0.97) and prilocaine hydrochloride/lidocaine hydrochloride (r^2 = 0.86) formulations respectively (Fig. 4).

DISCUSSION

From the *in vitro* validation results, it has been shown that the retrodialysis method provides reliable estimates of relative recovery values for both drugs, which are not signifi-

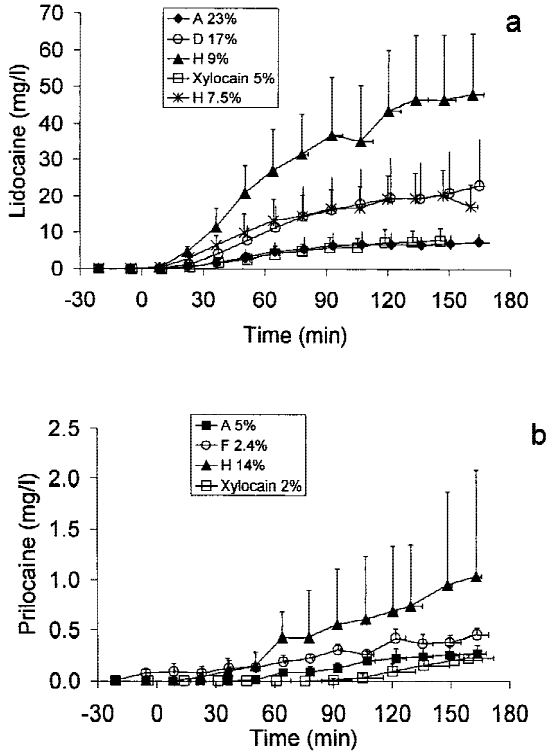

Fig. 3. Mean dermal concentration–time curves for (a) lidocaine and (b) prilocaine hydrochloride/lidocaine hydrochloride formulations (n = 3). Error bars indicate SD of the data points.

cantly different from true recovery. Furthermore, recovery values for lidocaine and prilocaine were not discernible ($P > 0.05$) and relative recovery was high for both drugs *in vivo.* This indicates an excellent potential for determination of cutaneous lidocaine and prilocaine levels by the microdialysis method, and that the respective drugs can be used as a reliable retrodialysis calibrator for the opposite drug during the experiments.

The presented pharmacokinetic model has been shown to provide excellent fits to concentration–time curves from cutaneous microdialysis, enabling assessment of apparent absorption rate and lag time. As for most data analysis methods, parameter estimates become more reliable, the smoother the obtained concentration-time curve is. However, even with very fluctuating curves, reliable estiamtes can be obtained with this method through minimisation of residual sum of squares by the Nelder-Mead simplex algorithm. Following lag time, dialysate concentrations of penetrated lidocaine were well above the limit of quantitation for the analysis, providing relative smooth coherent concentration-time curves with little deviation from ideal curve fit with the current pharmacokinetic model (Fig. 2a). However, dialysate concentations following application of prilocaine hydrochloride and lidocaine hydrochloride formulations were close to the limit of quantitation. In a few cases, this led to fluctuations in analyzed dialysate concentrations because of analytical errors, which impeded pharmacokinetic curve fitting slightly, but even though still providing robust estimates (Fig. 2b).

Individual microdialysis probe recovery varied not only as expected between experiments, but also within experiments with up to 21%, often decreasing slightly during the study. Thus, estimation of unbound tissue concentrations based on a fixed predetermined recovery level can lead to skewed values (15,18,21,22), and inferior curve fits. This correlates well with previous reports using the retrodialysis by calibrator method to correct for a decreasing recovery during the experiments in blood, lung and brain microdialysis in rats

Table II. Absorption Coefficient (k_{abs}) and Lag Time (t_{lag}) Estimates of Lidocaine (L) and Prilocaine Hydrochloride (P) Skin Penetration From Microemulsions and Xylocain® Cream/Gel, Based on *In Vivo* Microdialysis Concentration-Time Curves, with Recovery Correction[a]

Formulation	k_{abs} (μg/l/min)[b]	CV (%)	t_{lag} (min)	CV (%)
A 23% L	196 ± 183	94	24 ± 5	20
D 17% L	396 ± 243	61	25 ± 7	27
G 7.5% L	486 ± 374	77	16 ± 7	45
G 9% L	753 ± 378	50	15 ± 3	18
Xylocain® 5% L	89 ± 59.1	66	20 ± 6	28
A 5% P	6.0 ± 3.3	54	61 ± 21	35
E 2.4% P	5.6 ± 2.0	35	10 ± 18	173
G 14% P	8.9 ± 6.8	77	34 ± 9	28
Xylocain® 2% L[c]	5.2 ± 1.1	22	102 ± 12	12

[a] Mean ± SD (n = 3).
[b] n = 4.
[c] Lidocaine hydrochloride.

Table III. Absorption Coefficient (k_{abs}) and Lag Time (t_{lag}) Estimates of Lidocaine Skin Penetration from Microemulsions, Xylocain® Cream, and EMLA, Based on *In Vivo* Microdialysis Concentration–Time Curves Without Recovery Correction[a]

Formulation	k_{abs} (μg/l/min)	CV (%)	t_{lag} (min)	CV (%)
A 23% L	142 ± 117	82	21 ± 1	3
D 17% L	326 ± 184	56	24 ± 7	28
G 7.5% L	309 ± 171	55	15 ± 7	45
G 9% L	612 ± 282	46	15 ± 3	20
Xylocain® 5% L[b]	73 ± 49	67	20 ± 6	32
EMLA 2.5% L	230 ± 152	66	38 ± 15	38
EMLA 2.5% P[c]	217 ± 143	66	42 ± 18	41

[a] Mean ± SD (n = 3).
[b] n = 4.
[b] Prilocaine-free base.

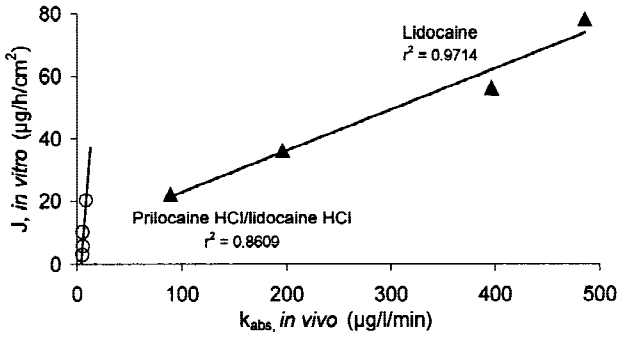

Fig. 4. Correlation between mean *in vivo* absorption coefficient (k_{abs}, n = 3), corrected for recovery, and *in vitro* flux (J, n = 3) of (▲) lidocaine formulations (data points from lowest to highest values: Xylocain® 5%, A 23% L, D 17% L, G 9% L, respectively) and (○) prilocaine hydrochloride/lidocaine hydrochloride formulations (data points from lowest to highest flux values: Xylocain® 2%, A 5% P, F 2.4% P, H 14% P, respectively). Solid line represents best linear fit.

experiment with several other drugs (15,18,21,22). This phenomenon has been suggested to be attributable to changes in the differential environment around the probe (15) or a reduction in probe recovery efficiency (18,22). A decrease in recovery of ^{3}H-glucose during the experiment using retrodialysis was also observed in a rat study monitoring (sub-) cutaneous glucose levels, by Lönnroth and collaborators (23,24). However, in contrast to previous reports, the authors suggested that this was due to accumulation of the calibrator around the probe, leading to artifact decrease in recovery, i.e., not a reflection of true relative recovery, and thus could not be used to correct estimated glucose levels during the experiment. The retrodialysis method requires sink conditions in the vicinity of the probe. Thus if the diffusion of the substance outside the probe is severely hampered, it is likely that a decreasing relative recovery may be attributable to this phenomenon (e.g., glucose loss into subcutaneous fatty tissues). This is not a likely explanation for the observations in e.g., blood microdialysis studies, however, Furthermore, the subcutaneous decreasing recovery of glucose observed in the rat study by Lönnroth and Strindberg, was not confirmed in a similar human retrodialysis study (23). A time-dependent relative recovery, hence, appears to be dependent on the diffusional/partition characteristics of the substance, the surrounding tissue and time interval studied (14,16).

In the present studies, the *in vivo* recovery was not substantially different from *in vitro* recovery, where outwards diffusion from the probe is unrestricted. Hence, it is assumed that sink conditions is maintained *in vivo,* and that the slight time-dependent decrease in recovery is due to changes in the diffusional and eliminating environment. Lidocaine has been demonstrated to be rapidly cleared from the dermis, but clearance may be significantly reduced by lowered capillary blood flow (25). It is generally acknowledged that implantation of microdialysis probes are associated with a temporarily increase in cutaneous blood perfusion, skin thickness and histamine release (20,24,26), which slowly subsides during the following hours. Additionally, minor local bleeding from disruption of the capillaries by implantation has been observed (24). In addition to biological variation in skin perfusion during the experiment (e.g., due to anaesthesia), these factors may be a plausible explanation for the frequently observed fluctuations and slight decrease in relative recovery in the present study. However, further investigations would be useful to elucidate the mechanisms of time-dependent relative recovery for various substances.

The large interindividual deviation in relative recovery is presumed to be attributable to alterations in the diffusional/eliminating environment in the vicinity of the probe. It was therefore expected that variability would decrease by recovery correction of concentration-time curves. However, no clear trend in CVs of pharmacokinetic parameters before and after recovery correction was observed and no significant correlation between elimination rate and relative recovery could be demonstrated. The statistical power of the investigation is hampered by the relative low number of replicates, though. Currently, a study using more replicates are being undertaken, which may clarify this relationship.

The results generally exhibit a larger CV of k_{abs} compared with lag time estimates. Variance of k_{abs} is mainly affected by individual barrier properties of the stratum corneum, V_d and possibly probe depth, whereas lag time mainly is affected by probe depth and stratum corneum barrier properties, indicating V_d as an additional source of variation. Thus it would be desirable to obtain reliable estimates of individual V_ds to further decrease variation of penetration rate estimates by the presented pharmacokinetic model.

The current microemulsion formulations have been assessed to increase the dermal penetration rates of the lipophilic model drug more than eight times compared to a conventional O/W-emulsion based vehicle and about 3-fold compared with an eutectic mixture based vehicle. The individual absorption coefficient ranks of the formulations correlated well with previous *in vitro* flux investigations with Franz diffusion cells (Fig. 4). Although absolute lidocaine concentration in the microemulsions was not the main determinant of the individual penetration ranks of the formulations, the increased thermodynamic activity of lidocaine in microemulsion G 9% compared to G 7.5%, resulted in a 55% larger absorption coefficient. The *in vitro* investigations (1) indicated that transdermal drug permeation from the microemulsions, was mainly related to molecular mobility of the drugs in the vehicle, which depended on internal structure of the microemulsion. The similarity of the microemulsion absorption coefficient ranks of the present *in vivo* investigations with the transdermal flux *in vitro,* indicates that drug mobility in the vehicle may also be one of the main determinates of dermal drug delivery rates from microemulsions *in vivo.*

The drug delivery results obtained for EMLA (Table 3) show that the free base of lidocaine and prilocaine have very similar pharmacokinetic profiles. Thus, it is assumed that the pharmacokinetic values of the prilocaine hydrochloride formulations are comparable to those of lidocaine hydrochloride (Table 2). This study demonstrated a significant decrease in lag time and indicated a mean increase in dermal penetration rate of the hydrophilic model drug in microemulsions, of almost 2-fold that of a commercial available hydrogel. The individual ranks of average absorption coefficients of the formulations with hydrophilic drugs also correspond well with *in vitro* flux results (1), except from Xylocain® 2% providing a significant lower transdermal flux than all microemulsions *in vitro* (Fig. 4).

The simplified controlled environment of *in vitro* permeation studies in Franz-type diffusion cells using excised skin as barrier membrane, is acknowledged as a robust preliminary method of estimating bioequivalence between various vehicles (27). The excellent relative correlation (Fig. 4) between cutaneous absorption found with the presented *in vivo* microdialysis model and the *in vitro* experiment (1), provides further evidence for the reliability of the apparent absorption rates estimates by the pharmacokinetic model. However, the significant deviating slopes of the *in vitro/vivo* correlations of lidocaine and prilocaine hydrochloride formulations (Fig. 4) shows that although *in vitro* results provide robust estimates of relative dermal penetration rate ranks between different formulations with the same drug, comparisons of drugs with different physical/chemical characteristics do not correlate to the *in vivo* situation. Together with the aim of assessing actual skin concentrations *in vivo,* this stresses the importance of obtaining of a reliable pharmacokinetic model to estimate cutaneous *in vivo* penetration on a routine basis, for which the microdialysis technique seems promising.

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