Influence of a Microemulsion Vehicle on Cutaneous Bioequivalence of a Lipophilic Model Drug Assessed by Microdialysis and Pharmacodynamics

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Purpose. The aim of the study was to investigate the cutaneous bioequivalence of a lipophilic model drug (lidocaine) applied in a novel topical microemulsion vehicle, compared to a conventional oil-in-water (O/W) emulsion, assessed by a pharmacokinetics microdialysis model and a pharmacodynamic method.

Methods. Dermal delivery of lidocaine was estimated by microdialysis in 8 volunteers. Absorption coefficients and lag times were determined by pharmacokinetic modelling of the microdialysis data. Subsequently, the anaesthetic effect of the treatments was assessed by mechanical stimuli using von Frey hairs in 12 volunteers.

Results. The microemulsion formulation increased the cutaneous absorption coefficient of lidocaine 2.9 times (95% confidence interval: 1.9/4.6) compared with the O/W emulsion-based cream. Also, lag time decreased from 110 ± 43 min to 87 ± 32 min ($P = 0.02$). The compartmental pharmacokinetic model provided an excellent fit of the concentration-time curves with reliable estimation of absorption coefficient and lag time. A significant anaesthetic effect was found for both active treatments compared to placebo ($P < 0.02$), but the effect did not diverge significantly between the two formulations.

Conclusions. The microemulsion vehicle can be applied to increase dermal drug delivery of lipophilic drugs in humans. The microdialysis technique combined with an appropriate pharmacokinetic model provides a high sensitivity in bioequivalence studies of topically applied substances.

KEY WORDS: microemulsion; microdialysis; pharmacokinetic; local anaesthetic; dermal; bioequivalence.

INTRODUCTION

Topical administration is an attractive choice for therapeutic agents, not only for systemic drug delivery, with avoidance of hepatic first-pass metabolism and increase of patient comfort compared with the oral and parenteral route, respectively, but particularly for targeted local drug delivery to the skin. However, to consider the cutaneous route viable, sufficiently high drug penetration rates into the skin have to be ensured in order to reach therapeutic levels in the targeted organ.

The application of substances in microemulsion vehicles (1) have *in vitro* and *in vivo* in animals been indicated to increase transdermal and dermal delivery for lipophilic and hydrophilic drugs compared with conventional topical vehicles, depending on composition and structure of the microemulsion (2–9). However, the clinical potential of increasing dermal drug delivery using microemulsions is yet relatively unexplored. The potential of increasing drug delivery by application in microemulsions appears to be related to the large concentration gradient from the vehicle to the skin, enabled by the excellent solubility properties (2,3,6,9). Also, it has been suggested that the interaction between the rigid lamellar bilayer lipid structure of the stratum corneum and the surfactant system or oil may facilitate drug penetration (8–10).

The microdialysis technique has been shown to provide reliable estimates of skin absorption of exogenous compounds (2,11–13) and uniquely enables assessment of unbound extracellular compound levels directly in the target organ, i.e., the dermis layer of the skin for dermal drug delivery. Microdialysis have in rats been demonstrated to be a very promising technique to assess cutaneous bioequivalence of pharmaceutical formulations (2,4,14,15), but this potential of the technique in humans and the relevance to the clinic has yet to be realized. However, a relatively large inter- and intraindividual variability of cutaneous drug levels assessed by microdialysis has been observed (12,16), which may hamper bioequivalence studies because vehicels without penetration enhancers typically do not affect the penetration rate of a drug more than 10- to 20-fold (17). A recent microdialysis study in rats (4) has indicated that variance in cutaneous microdialysis experiments may be substantially reduced by individually monitoring relative recovery of the substance and by differentiation of the pharmacokinetic parameters through compartmental modelling of the concentration-time curves.

The objective of this study was to evaluate the cutaneous bioequivalence of a lipophilic model drug (lidocaine) applied topically in a novel low-irritant microemulsion vehicle, compared with a conventional marketed oil-in-water (O/W) emulsion, in healthy volunteers. Furthermore, the microdialysis technique was compared with a pharmacodynamic assessment method for bioequivalence studies, in terms of precision and sensitivity.

MATERIALS AND METHODS

Subjects

The study was performed in two parts. The pharmacokinetics of lidocaine after topical administration were assessed in an open study in eight healthy male volunteers (age: 19–30 years) using microdialysis. The pharmacodynamics of the lidocaine formulations were evaluated in 12 (four female, eight male) volunteers (age: 19–30 years) using a placebo-controlled design. The subjects participating in the pharmacokinetic study were included in the pharmacodynamic study, with a minimum recovery period of three days in between. Subject demographics are summarized in Table I.

Subjects were given a detailed description of the study and written consent was obtained. The study was approved by the ethics committee of Leiden University Medical Centre, and was conducted according to the principles of the "Declaration of Helsinki" and in accordance with the Guideline for Good Clinical Practice.

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Table I. Subject Demographics

	Pharmacokinetic part (n = 8 males)		Pharmacodynamic part (n = 8 males, n = 4 females)	
	Average	Range	Average	Range
Age (years)	22	19–30	22	19–30
Height (cm)	183	177–191	180	167–191
Weight (kg)	78	54–99	79	54–99

Treatments

The pharmacokinetic part consisted of two treatments, consisting of Xylocain 5% (w/w) cream (lidocaine) and a microemulsion (basic composition: 65% sterile water, 3% isostearyle isostearate, 24% Labrasol and 8% Plurol Isostearique w/w), characterized in recent studies (3,4), containing 7.5% (w/w) lidocaine. The pharmacodynamic part additionally included a placebo microemulsion with the same basic composition, without lidocaine. At each application site, 2 ml of the current formulation was administered for a 4-h period, using a randomized double-blinded design.

Labrasol® (Caprylocaproyl Macrogolglycerides), Plurol Isostearique® (polyglyceryl isostearate), and isostearylic isostearate (>92% purity) (Gattefossé, Lyon, France) were products of Gattefossé S.A. (Lyon, France) and were obtained from Bionord A/S (Hellerup, Denmark). The same batch of the microemulsion components was used throughout all experiments. Lidocaine was purchased from Bufa BV, Uitgeest, The Netherlands and prilocaine HCl (Citanest 2%) from Astra, Astra Pharmaceutica BV, Zoetermeer, The Netherlands Xylocain® 5% cream (lidocaine) (Astra, Södertälje, Sweden) is a commercial product. Sterile, distilled water was used throughout the experiments.

Study Days

The pharmacokinetic microdialysis study was performed on a single occasion. The subject was placed in semi-recumbent position. In an area of 10 × 5 cm in the center of the left volar forearm, hairs were removed with an electrical hairclipper. At each of the two application sites (7 cm apart), two microdialysis probe entrance and exit points were marked and the area was disinfected. At each application site, two 22-G guide cannulas were implanted, 5 mm apart in the dermis, at a length of 30 mm, and resurfacing through exit punctures. Probe implantation was done without anaesthesia under sterile conditions. The cannulas were placed as superficially as possible. Through the tip of each cannula, a microdialysis probe was inserted and the needle retracted, leaving the probe fiber implanted in the skin. Subsequently, a 7-cm polythene 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 fiber site, a cylindrical polyethylene application chamber (22 mm ID, 3 ml volume) was glued to the skin with Histoacryl glue (enbucrilate, Braun Surgical GmbH, Melsungen, Germany). The subject was followed a minimum recovery period of 90 min after probe implantation to diminish skin reactions (i.e., increased blood flow and histamine release) (18,19) before onset of the experiment. After a 15-min baseline sampling of dialysate, 2 ml of the current formulation was injected into the application chamber and the chamber sealed with Tegaderm® (3M, Denmark). Dialysate sampling was continued for 4 h, replacing collection vials every 15 min. The samples were assayed for lidocaine and prilocaine content by high-performance liquid chromatography (HPLC) after the experiment within 24 h. Time points were calculated as the midpoint between sampling intervals and corrected for lag time of the perfusate from the microdialysis site to the probe outlet. At the end of the microdialysis sampling period, probe depth was measured using ultrasound (Toshiba Sonolayer SSA-250A, Toshiba Corporation, Japan equipped with a 7.5 MHz probe).

Microdialysis System

The microdialysis system consisted of a CMA/100 microinjection pump (CMA/Microdialysis AB, Stockholm, Sweden) equipped with 2.5 ml of Exmire microsyringes (ITO Corporation, Fuji, Japan). A sterile aqueous saline solution (Na^+ 165.4 mM, PO_4^{3-} 47.2 mM, Cl^- 110.2 mM) buffered at pH 6.5, containing prilocaine (20 mg/l) as recovery calibrator, was used as perfusate at a flow rate of 1.2 μl/min. Custom-made microdialysis probes with a linear design (4), based on a single 30-mm dialysis fiber (208-μm inner diameter (ID), 216-μm outer diameter, 2 kDa molecular weight cut off) from a dialysator (Gambro GFS +12, Gambro Dialysaten, Hechingen, Germany) were used. Probes were sterilized with ethyleneoxide gas prior to use. Relative recovery (RR) was calculated using equation 1, by measuring loss of prilocaine from the perfusate according to the retrodialysis method (20).

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

Pharmacodynamic Assessment of Anaesthesia

The studies were performed on a single occasion in a quiet room at fixed temperature (20–22°C). Von Frey hairs (21,22) with three filament thicknesses (#11: 0.35 mm, #15: 0.55 mm, and #19: 1.0 mm) (Somedic Sales AB, Hörby, Sweden) corresponding to an average prod force of 1.7, 17, and 110 g, respectively, were used. Before the study onset, a short refreshment of the pain sensation and scoring on a visual analogue scale (VAS) was performed.

The subject was placed in semi-recumbent position, and in an area of 20 × 5 cm in the center of the left volar forearm, hairs were removed with an electrical hairclipper. At each of the three application sites (5 cm apart) a cylindric polyethylene application chamber (22 mm ID, 3 ml volume) was glued to the skin with Histoacryl glue. Prodding of the hairs was done by placing the filament in the center of a circular 1-cm hole on top of the application chamber, in a vertical angle to the skin and slowly applying pressure to the handle shaft in direction of the skin. Pressure was exerted until the filament described a half-arch with a 30-degree angle between the end piece of the filament and the initial vertical line. Duration of the suppression was approximately 1 s, followed by 1 s static position and a 1 s retraction period. The volunteers were instructed to close their eyes before the prodding and subsequently score the evoked pain on a continuous 100-mm VAS, where minimum (0 mm) was no sensation and maximum (100

mm) was unbearable pain. The three hairs were prodded once at each application site in a randomized, single-blinded fashion, every 15 min (except at formulation applicaton; t = 0) during a 4.5 h time period starting at t = –30 min. At onset of the treatments (t = 0), 2 ml of the current formulation was injected into the appropriate application chamber and the chamber sealed with Tegaderm® (3M, Denmark). A small crack was induced in the Tegaderm® to allow penetration of the hairs into the chambers. Anaesthetic effect of the treatments was calculated as area under the VAS scores versus time curve divided by the corresponding time period (AUE) resulting in a weighted average pain score.

HPLC Assay

Lidocaine and prilocaine were quantified using an HPLC system (Milford, MA, USA) consisting of a Waters 712 autosampler (7 μl sample injections), Waters 515 pump operating at 0.4 ml/min, Waters 490 UV-detector (205 nm) and a Shimadzu C-R3A integrator (Tokyo, Japan). Analytes were separated by a narrowbore Waters Symmetry Shield™ C-18 column (5 μm, 150 × 2.1 mm) 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 7.0. 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 detection was 0.14 mg/l and 0.15 mg/l, coefficient of variation (CV) was 12.1% and 12.9% at 1 mg/l for prilocaine and lidocaine, respectively. CV was 0.75% at 25 mg/l for prilocaine and 0.27% at 60 mg/l for lidocaine. Solvents were of HPLC grade, and all other chemicals were of analytical grade and used as received.

Pharmacokinetic and Statistical Analysis

Assayed lidocaine concentrations in the dialysate samples were corrected for estimated recovery at each sampling interval, unless stated otherwise. Pharmacokinetic analysis was performed by PC-compatible software WinNonlin™ © version 2.1 (Pharsight Corporation, Mountain View, CA, USA). Concentration-time curves of lidocaine were fitted to a zero order absorption (R_0), one compartment (V_d), and first order elimination (k_e) model, including a lag time (t_{lag}), according to:

$$C = \frac{k_{abs}}{k_e}(1 - e^{-k_e(t - t_{lag})}) \quad (2)$$

where k_{abs} is 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 minimization method. The absorption coefficient k_{abs} estimates the initial rate of concentration change (at time = t_{lag}). In order to estimate total drug exposure the AUC over the entire application period (4 h) was calculated using non-compartmental analysis.

The parameters are presented as mean ± standard deviation (SD), unless stated otherwise. Inter-individual variability of the pharmacokinetic parameters was calculated as a weighted average of the mean CV from the microemulsion and Xylocain treatments, respectively. Intra-individual variability was estimated as the mean of the calculated CVs of the pharmacokinetic parameters from the two probes for each treatment. Paired two-tailed Student's *t*-tests were used for statistical analysis of both the pharmacodynamic and pharmacokinetic parameters (using the average value of the two probes for the pharmacokinetic parameters) after log-transformation. Contrasts were back-transformed with their 95% confidence intervals resulting in estimates of the ratio or percentage decrease. *P*-values <0.05 were considered statistically significant.

RESULTS

Pharmacokinetic Study

The microdialysis implantation procedure was well tolerated by all subjects. The reported discomfort ranged from not noteworthy, to moderately painful, and comparable with the pain associated with venipuncture. Determination of the exact location of the microdialysis fibers was problematic because of the low frequency (resolution) of the available ultrasound apparatus, and probe depth was therefore not registered for the majority of the subjects. However, the expected probe placement in the lower dermis layer of the skin was confirmed in three subjects (one probe each) at approximately 1 mm from the skin surface.

Relative Recovery

The retrodialysis by calibrator method using prilocaine as calibrator has *in vitro* and *in vivo* in rats been shown to provide reliable and concentration independent estimates of lidocaine relative recovery (4). RR during sampling periods varied between 56–95% during the study. Furthermore, recovery fluctuations were also observed within the experiments for each probe, occasionally with a slightly decreasing recovery during the experiment (e.g., Fig. 1). The average relative recovery fluctuation within experiments was 12.4 ± 4.7% (n = 31) with a maximum fluctuation of 23.1% during a single experiment.

Bioequivalence

The pharmacokinetic model provided excellent fits to all microdialysis concentration-time curves (average and worst-case curve fit exemplified in Fig. 2; [correlation between observed and predicted concentrations: (a) 0.994 and (b) 0.985]).

The estimated absorption coefficients and lag times of lidocaine, obtained using the fitting procedure (Eq. 2), are collected in Tables II and III. It should be noted, that due to

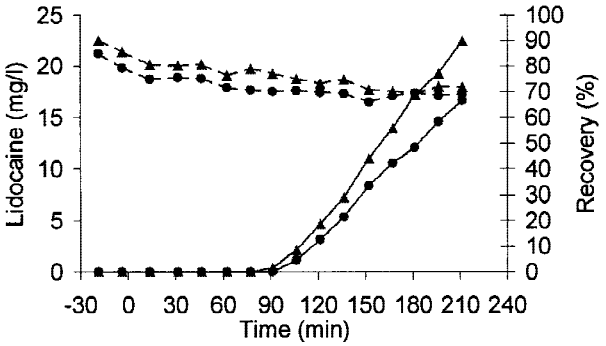

Fig. 1. Example of recovery fluctuation during a microdialysis experiment (Xylocain, subject 8). Solid lines represent lidocaine penetration and dotted lines calibrator recovery determined from probe A (▲) and B (●).

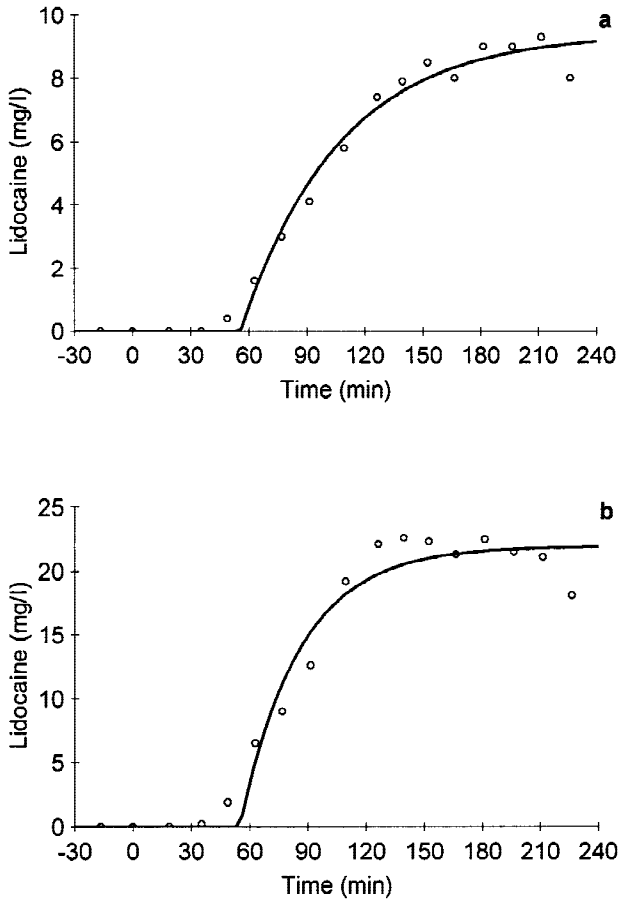

Fig. 2. Typical (a: Xylocain, subject 1, probe A) and worst-case (b: Microemulsion, subject 1, probe A) pharmacokinetic curve fit to cutaneous microdialysis concentration-time curves. ○ represents actual dialysate concentrations (corrected for recovery) and solid line represents predicted concentrations based on the pharmacokinetic model.

Table II. Absorption Coefficient (k_{abs}, μg/l/min) Estimates of Lidocaine Skin Penetration from Microemulsion and Xylocain Using Paired Microdialysis Probes (A and B) at Each Application Site

	Microemulsion			Xylocain		
Subject	Probe A	Probe B	Average	Probe A	Probe B	Average
1	711	354	533	184	125	154
2	53	205	(129)	(22)[a]	(20)[b]	(21)
3	187	201	194	200	68	134
4	363	161	262	62	49	56
5	256	405	330	224	107	165
6	311	444	378	–[c]	149	149
7	572	440	506	222	156	189
8	994	1123	1058	200	159	179
Mean ± SD			466 ± 288 (424 ± 292)[d]			147 ± 44 (131 ± 60)[d]

[a] Estimate based on two data points.
[b] Estimate based on three data poins.
[c] Probe failed during the experiment.
[d] Subject 2 included.

Table III. Lag Time (t_{lag}, min) Estimates of Lidocaine Skin Penetration from Microemulsion and Xylocain Using Paired Microdialysis Probes (A and B) at Each Application Site

	Microemulsion			Xylocain		
Subject	Probe A	Probe B	Average	Probe A	Probe B	Average
1	55	45	50	56	58	57
2	147	139	143	213	196	204
3	128	116	122	125	135	130
4	86	87	86	102	102	102
5	71	71	71	94	85	89
6	86	83	85	–[a]	100	100
7	87	87	87	100	94	97
8	50	51	51	97	100	99
Mean ± SD			87 ± 32			110 ± 43

[a] Probe failed during the experiment.

the very long lag time of lidocaine applied in Xylocain for subject 2, only two and one lidocaine dialysate concentrations from each probe, respectively, were above the analytical limit of detection. This was considered insufficient for estimation of an absorption coefficient, and subject 2 was thus excluded from further data analysis of this parameter. The microemulsion vehicle increased the mean absorption coefficient of lidocaine (466 ± 288 μg/l/min) into the skin 2.9-fold (95% CI: 1.9/4.6), compared to the conventional O/W emulsion (147 ± 44 μg/l/min). Additionally, the mean lag time of lidocaine entering the dermis layer of the skin was significantly reduced from 110 ± 43 min to 87 ± 32 min ($p = 0.02$) by the microemulsion. Mean AUC_{0-4h} for the time-concentration curves during application of lidocaine in the microemulsion was 2900 ± 2690 mg/l, which was 4.3 times higher (95% CI: 1.5/12.5) than the conventional emulsion (AUC_{0-4h} = 867 ± 488 mg/l).

Variability

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 inter-individual CV (38%). Also mean CV of absorption coefficients was substantially lower intra-individually (30%), in comparison to inter-individually (46%).

To investigate the influence of dermal elimination on relative recovery variation between probes, mean relative recovery during the experiment was plotted as a function of estimated elimination rate of lidocaine (from concentration-time curves without correction for relative recovery) at each probe site (Fig. 3). More reliable estimates of elimination rate from the pharmacokinetic model were generally obtained for treatments with shorter lag times where concentration-time contained substantial elimination information (approaching a steady-state level). Therefore, only data for experiments with lag time <95 min (n = 16) were included in this comparison. This study indicated a significant linear correlation ($r^2 = 0.51$; $p < 0.002$) between elimination rates and mean relative recovery (Fig. 3).

Individual recovery correction of the concentration-time curves reduced CV of the pharmacokinetic parameters, compared to non-corrected data (not shown). CV of mean esti-

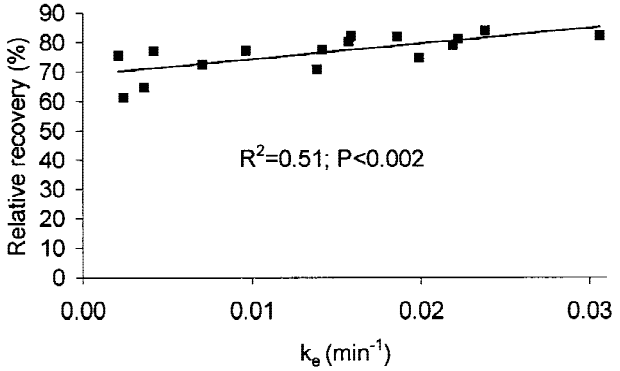

Fig. 3. Correlation between mean relative recovery during experiments and estimated elimination rate of lidocaine in the skin (k_e) for microdialysis probe implantation sites with an estimated lag time <95 min (n = 16).

mated absorption coefficient of lidocaine from Xylocain 5% was reduced from 39% to 30% and CV of estimated lag time was additionally slightly reduced from 40% to 39%. Similarly, CV of estimated absorption coefficient of lidocaine from the microemulsion formulation (n = 7) was reduced from 66% to 62% and CV of estimated lag time from 39% to 37%.

Pharmacodynamic Assessment

A significant difference was found between the baseline scores ($p < 0.001$) of the three filament thicknesses (#11: 4.9 ± 4.5, #15: 21.5 ± 10.3, and #19: 40.8 ± 17.3), demonstrating the gradual increase in pain sensation with filament thickness, ranging from faintly painful (#11) to moderately painful (#19). Mean pain scores at the application site of the placebo microemulsion, did not diverge significantly from initial baseline values during the experiments for any of the von Frey hairs. However, pain perception decreased following active treatment (Fig. 4). Summaries of the AUEs of the VAS pain scores during application of active microemulsion, Xylocain, and placebo microemulsion, respectively, are presented in Table IV. For all three pain levels, a statistically significant anaesthetic effect was found for both Xylocain and the active microemulsion, compared to the placebo treatment. During active treatments with microemulsion/Xylocain mean AUE was reduced by 41/39%, 26/23%, and 19/18% ($p < 0.02$) for mechanical stimulation with von Frey hair #11, #15, and #19, respectively. However, although mean AUEs obtained with the active microemulsion were slightly lower compared to those of Xylocain for all three filaments, no statistically significant difference was demonstrated between the two treatments ($p > 0.77$). No linear correlation was found between AUEs and any of the pharmacokinetic parameters (k_{abs}, t_{lag}, or AUC), for the subjects participating in both the pharmacokinetic and pharmacodynamic study ($r^2 < 0.22$). However, the graphs indicate that the approximate onset of anaesthesia (75–100 min) corresponds well with the estimated lag times in the pharmacokinetic study.

DISCUSSION

The microdialysis study demonstrated a significant decrease in lag time and a significant increase in rate of absorption of a lipophilic model drug into the skin when applied in the studied microemulsion vehicle, compared with a conventional O/W emulsion. This corresponded to a more than four-

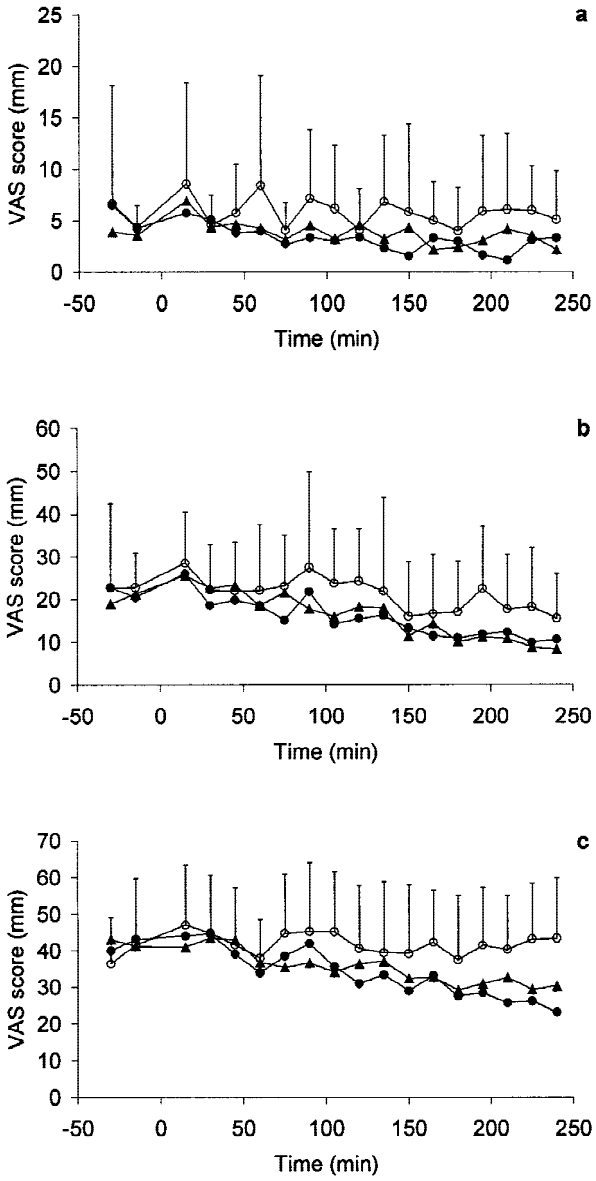

Fig. 4. Average VAS pain scores vs. time (n = 12) from mechanical stimuli with von Frey hair (a) #11, (b) #15, and (c) #19 during application of (●) microemulsion (7.5% lidocaine), (▲) Xylocain (5% lidocaine) and (○) placebo microemulsion, respectively. Error bars represent SD and are omitted from the microemulsion and Xylocain graphs to increase clarity.

fold increase in total amount of lidocaine delivered to the dermis when applied in the microemulsion vehicle compared to the emulsion, which is mainly attributable to the increase in absorption rate. Previous studies have indicated that the cutaneous drug delivery from the tested microemulsion vehicle is increased, mainly due to the large drug solubility in the vehicle (which creates a large concentration gradient towards the skin), combined with a high molecular diffusivity of lidocaine in this vehicle, due to the dynamic nature of microemulsion structures (2).

A large variability of dermal drug levels following topical application, which has been demonstrated in earlier microdialysis studies (4,12,16), was also observed in the present study. The source of variability in cutaneous drug delivery may arise from both inter- and intra-individual differences in absorption rate

Table IV. Time Averaged (0–4 h) AUE (mm) of Pharmacodynamic VAS Pain Scores for Mechanical Stimuli with von Frey Hair #11, #15, and #19 During Application of Microemulsion (7.5% lidocaine), Xylocain (5% lidocaine), and Placebo Microemulsion (n = 12), Respectively

	Mean AUE ± SD			Percentage decrease (95% confidence interval)		
Hair size	Microemulsion	Xylocain	Placebo	Microemulsion vs Placebo	Xylocain vs Placebo	Microemulsion vs Xylocain
von Frey 11	3.4 ± 1.8	3.9 ± 3.5	5.8 ± 3.7	41 (16/58) $P = 0.006$	39 (13/57) $P = 0.010$	4 (–27/27) $P = 0.770$
von Frey 15	16.4 ± 7.9	17.1 ± 9.9	21.7 ± 11.0	26 (5/42) $P = 0.023$	23 (14/32) $P < 0.001$	3 (–23/23) $P = 0.786$
von Frey 19	34.8 ± 14.2	36.0 ± 17.5	41.9 ± 13.7	19 (9/29) $P = 0.003$	18 (8/27) $P = 0.003$	2 (–12/14) $P = 0.769$

and lag time (skin barrier function), distribution and elimination of the drug (metabolism and diffusion rate to the systemic circulation) and by differences in probe implantation (depth and tissue properties affecting relative recovery of the drug).

The presented pharmacokinetic model applied in this study appears to enable reliable estimation of absorption coefficients and lag times of topically applied drugs using drug levels obtained by microdialysis. The model accounts for the influence of deviating elimination rate from the microdialysis sites within and between subjects, which leads to a decrease in variance of pharmacokinetic parameters compared to non-compartmental comparators (e.g. C_{max}, AUC). In the present study, CV was substantially lowered from parameters estimated by the pharmacokinetic model (k_{abs}-microemulsion: 62%, t_{lag}-microemulsion: 37% and k_{abs}-Xylocain: 30%, t_{lag}-Xylocain: 39%) compared to AUCs (AUC-microemulsion: 91% and AUC-Xylocain: 63%) for both vehicles. The increase in precision may extend the relevance of the technique for bioequivalence studies of topical formulations where absorption rates do not differ more than one order of magnitude.

Relative recovery of lidocaine, as determined by the retrodialysis by calibrator method, showed that recovery does fluctuate, not only between subjects, but also during the experiment. Approximately 50% of the variation in relative recovery between probes was indicated to be attributable to differences in elimination rate in the dermis (Fig. 3). This suggests that the dermal diffusivity of the drug (indicated by the elimination rate) is one of the main variables of relative recovery *in vivo.* A further reduction in variability of assessed pharmacokinetic parameters was demonstrated by individual correction for relative recovery of each probe, increasing reproducibility of the microdialysis technique, even though deviations in elimination rate were already accounted for by the pharmacokinetic model.

The influence of probe depth on variability of cutaneous microdialysis data has been a subject of discussion in the literature and appears to depend on diffusion and elimination characteristics of the investigated drug, and the displacement distances of the probes in the skin (2). Although most authors have not found a correlation between drug levels in the skin and probe depth (11–13), an association has been indicated between nicotine steady state concentration and probe depths of 1–10 mm from the skin surface (23). In the present study, a relatively low intra-individual CV for the pharmacokinetic parameters of the paired probes under each application site was observed, which implies that probe implantation (i.e., probe depth) is not a major source of variation. The substantial larger inter-individual CV of the pharmacokinetic parameters, compared to the intra-individual CVs, indicate that inter-individual differences in skin barrier function is one of the major contributors to the observed variability in cutaneous drug levels after topical application.

Rat skin is generally considered to be 2–5 times more permeable than human skin *in vitro* (17), which hampers quantitative prediction of cutaneous drug delivery in humans from rat studies. Interestingly, the absorption coefficients of lidocaine from the two test formulations found in the present study are not significantly different from the absorption coefficients observed in an experimentally similar cutaneous microdialysis study in Wistar rats (4) (Xylocain 5%: 89 ± 59 μg/l/min; microemulsion 7.5%: 486 ± 374 μg/l/min). However, dermal lag time for both formulations was approximately 5–6 times shorter in rats (Xylocain 5%: 20 ± 6 min; microemulsion 7.5%: 16 ± 7 min). These findings indicate that the diffusion rate of the model drug in the rate-limiting barrier layer of the skin is similar in rats and humans *in vivo* but the diffusion pathway is longer in humans. Thus, besides the good qualitative agreement between cutaneous bioequivalence in rats and humans, this study indicates that it may be possible to quantitatively predict the absorption rate of novel topical formulations in humans from rat studies using the microdialysis technique. However, not all substances display this excellent agreement (24), and more studies would be needed to establish the *in vivo* correlation between permeability of rat and human skin.

The pharmacodynamic study has demonstrated a statistical significant anaesthetic effect of the active microemulsion and Xylocain compared with placebo microemulsion, which was inversely related to the level of pain stimulation (filament thickness). However, the anaesthetic effect of the active microemulsion was not significantly different from that of Xylocain despite a shorter lag time and larger absorption coefficient of the former. It may be speculated that this is due to an already maximum effect at low lidocaine tissue concentration on the action potential of C- and Aδ-fibers (which are responsible for pain sensation). However, using the saphenous nerve of cats, a dose-dependent depressed action potential of the C- and Aδ-fibers at high lidocaine concentrations has been demonstrated previously (25). This study indicates that the concentration dependency of the anaesthetic effect of lidocaine in the assessed concentration range is relatively small, however. Appliation of a model drug with higher efficacy in the microemulsion vehicle would presumably result in larger differences in pharmacodynamic response, correspond-

ing to the 4.3-fold increase in the AUC. The minor pharmacodynamic differences may also be attributable to the large variability and lack of sensitivity of the von Frey hair method. It is generally accepted that pharmacodynamic assessments are associated with larger variability compared with pharmacokinetic studies, particularly when the assessed parameters are based on subjective evaluations. Furthermore, the mechanosensitive fields of the C-fibers are randomly distributed in small branches of typically 6–150 mm^2 in the human skin (26), increasing variance between application sites. The pain sensation at the placebo application site did not diverge significantly from initial baseline value for any of three filaments during the experiments (Fig. 4), indicating that no tolerance or hyperalgesia was induced by the mechanical stimuli. The unchanged standard deviation of the mean pain scores during the experiment suggests that the subjects were trained adequately in pain sensation and VAS scoring. Therefore, it is likely that the main variance can be attributed to the von Frey hair method itself. This emphasizes the significance of developing a sensitive and reliable technique, like the presented microdialysis model, to assess differences in dermal bioequivalence of topical formulations *in vivo.*

The clinical relevance of topical microemulsion formulations also relies on low dermatological irritancy. *In vitro* studies using rat skin have shown that the barrier function of the stratum corneum is not significantly degraded by 20-h applications of a microemulsion with larger surfactant and oil content using the same components as the present microemulsion (3). A clinical study using a microemulsion vehicle based on the same surfactant system (Labrasol/Plurol Isostearique) used in this study additionally confirmed that skin barrier function evaluated by transepidermal water loss was not affected by a 3-h application period (8).

Using a small study population in combination with the single occasion paired study design, the pharmacokinetic microdialysis model enables differentiation between topical formulations, which do not display vast differences in cutaneous lag times and penetration rates. Together with the ease of formulation and the thermodynamic stability, the excellent dermal drug delivery properties demonstrated in this study, makes the microemulsion vehicle an attractive choice for future topical formulation.

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REFERENCES

1. T. P. Hoar and J. H. Schulman. Transparent water in oil dispersions: Oleopathic hydromicelle. *Nature* **152**:102 (1943).
2. M. Kreilgaard. Cutaneous drug delivery potential of microemulsion vehicles—Application of a pharmacokinetic microdialysis model to assess skin penetration. [Ph.D. thesis]. The Royal Danish School of Pharmacy, Copenhagen, Denmark. (2000).
3. M. Kreilgaard, E. J. Pedersen, and J. W. Jaroszewski. NMR characterisation and transdermal drug delivery potential of microemulsion systems. *J. Control. Release* **69**:421–433 (2000).
4. M. Kreilgaard. Dermal pharmacokinetics of microemulsion formulations determined by in vivo microdialysis. *Pharm. Res.* **18**: 367–374 (2001).
5. L. Boltri, S. Morel, M. Trotta, and M. R. Gasco. In vitro transdermal permeation of nifedipine from thickened microemulsions. *J. Pharm. Belg.* **49**:315–320 (1994).
6. K. Kriwet and C. C. Müller-Goymann. Diclofenac release from phospholipid drug systems and permeation through excised human stratum corneum. *Int. J. Pharm.* **125**:231–242 (1995).
7. F. P. Bonina, L. Montenegro, N. Scrofani, E. Esposito, R. Cortesi, E. Menegatti, and C. Nastruzzi. Effects of phospholipid based formulations on in vitro and in vivo percutaneous absorption of methyl nicotinate. *J. Control. Release* **34**:53–63 (1995).
8. M. B. Delgado-Charro, G. Iglesias-Vilas, J. Blanco-Mendez, M. A. López-Quintela, and R. H. Guy. Delivery of a hydrophilic solute through the skin from novel microemulsion systems. *Eur. J. Pharm. Biopharm.* **43**:37–42 (1997).
9. F. Dreher, P. Walde, P. Walther, and E. Wehrli. Interaction of a lecithin microemulsion gel with human stratum corneum and its effect on transdermal transport. *J. Control. Release* **45**:131–140 (1997).
10. U. Schmalfuss, R. Neubert, and W. Wohlrab. Modification of drug penetration into human skin using microemulsions. *J. Control. Release* **46**:279–285 (1997).
11. L. Hegemann, C. Forstinger, B. Partsch, I. Lagler, and K. Wolff. Microdialysis in cutaneous pharmacology: Kinetic analysis of transdermally delivered nicotine. *J. Invest. Dermatol.* **104**:839–843 (1995).
12. M. Müller, H. Mascher, C. Kikuta, S. Schafer, M. Brunner, G. Dorner, and H. G. Eichler. Diclofenac concentrations in defined tissue layers after topical administration. *Clin. Pharmacol. Ther.* **62**:293–299 (1997).
13. E. Benfeldt, J. Serup, and T. Menne. Effect of barrier perturbation on cutaneous salicylic acid penetration in human skin: In vivo pharmacokinetics using microdialysis and non-invasive quantification of barrier function. *Br. J. Dermatol.* **140**:739–748 (1999).
14. K. Matsuyama, M. Nakashima, M. Ichikawa, T. Yano, S. Satoh, and S. Goto. In vivo microdialysis for the transdermal absorption of valproate in rats. *Biol. Pharm. Bull.* **17**:1395–1398 (1994).
15. M. Nakashima, M. F. Zhao, H. Ohya, M. Sakurai, H. Sasaki, K. Matsuyama, and M. Ichikawa. Evaluation of in vivo transdermal absorption of cyclosporin with absorption enhancer using intradermal microdialysis in rats. *J. Pharm. Pharmacol.* **48**:1143–1146 (1996).
16. J. M. Ault, C. M. Riley, N. M. Meltzer, and C. E. Lunte. Dermal microdialysis sampling in vivo. *Pharm. Res.* **11**:1631–1639 (1994).
17. H. Schaefer and T. E. Redelmeier. *Skin Barrier: Principles of Percutaneous Absorption,* Karger, Basel, 1996.
18. C. Anderson, T. Andersson, and K. Wardell. Changes in skin circulation after insertion of a microdialysis probe visualized by laser Doppler perfusion imaging. *J. Invest. Dermatol.* **102**:807–811 (1994).
19. L. Groth and J. Serup. Cutaneous microdialysis in man: Effects of needle insertion trauma and anaesthesia on skin perfusion, erythema and skin thickness. *Acta Derm. Venereol.* **78**:5–9 (1998).
20. S. L. Wong, Y. Wang, and R. J. Sawchuk. Analysis of zidovudine distribution to specific regions in rabbit brain using microdialysis. *Pharm. Res.* **9**:332–338 (1992).
21. M. von Frey. Untersuchungen über die Sinnesfunctionen der menschilichen Haut: Druckempfindung und Schmerz. *Abhl. sächs. Gesell. Wiss. math.-phys.* **23**:175–266 (1896).
22. D. P. Barker and N. Rutter. Lignocaine ointment and local anaesthesia in preterm infants. *Arch. Dis. Child* **72**:F203–F204 (1995).
23. M. Müller, R. Schmid, O. Wagner, B. Osten, H. Shayganfar, and H. G. Eichler. In vivo characterization of transdermal drug transport by microdialysis. *J. Control. Release* **37**:49–57 (1995).
24. E. Benfeldt. In vivo microdialysis for the investigation of drug levels in the dermis and the effect of barrier perturbation on cutaneous drug penetration—Studies in hairless rats and human subjects. *Acta Derm. Venereol. Suppl.* **206**:7–54 (1999).
25. R. H. de Jong and R. A. Nace. Nerve impulse conduction during intravenous lidocaine injection. *Anesthesiology* **29**:22–28 (1968).
26. J. van Hees and J. Gybels. C nociceptor activity in human nerve during painful and non painful skin stimulation. *J. Neurol. Neurosurg. Psychiatry* **44**:600–607 (1981).