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DSC MEASUREMENTS ON FULL THICKNESS MICE SKIN
An additional tool to investigate permeation enhancement of highly lipophilic drugs

R. Schiller[1*], *A. P. Funke*[2] *and C. Günther*[3]

[1]Analytical Development/Phys. Chemistry, Schering AG, 13342 Berlin, Germany
[2]Pharmaceutical Development, Oral Dosage Forms, Schering AG, 13342 Berlin, Germany
[3]Preclinical Pharmacokinetics, Schering AG, 13342 Berlin, Germany

Abstract

Highly lipophilic basic drugs, the antiestrogens AE 1 and AE 2 shall be delivered transdermally. DSC as an additional tool in combination with classic investigation techniques should be used to clarify permeation enhancement. Skin treatment with pure solvents, polyethyleneglycol (PG) and dimethylisosorbide (DMI), slightly changed the phase transition temperatures. Formulations containing lauric acid markedly shifted these transitions to lower temperatures, indicating a lipid-fluidising action of lauric acid. In those cases an additional endothermic peak was observed around 40°C, which is attributed to the melting of crystalline lauric acid. Since the DSC program started at –20°C, it is very likely that lauric acid in the skin samples crystallized. A formulation of polyethyleneglycol and lauric acid leads to significantly higher deposition of lauric acid into the skin, in opposition to dimethylisosorbide/lauric acid formulation. These findings correspond to the results from our in-vitro permeation studies, where a significantly higher transdermal steady-state flux of lauric acid from polyethyleneglycol-formulation in comparison to dimethylisosorbide-formulation was observed. By this unique combination of polyethyleneglycol and lauric acid, the barrier is obviously modified in a way, which allows the highly lipophilic antiestrogens to permeate easily through the skin. So, from this formulation steady-state fluxes of AE 1 were observed, representing approximately the same value compared to the unhindered permeation through skin without stratum corneum. The grade of temperature shift on the skin lipids to lower temperatures can be correlated with softening effects and the enhancement potential of the formulation.

Keywords: antiestrogens, DSC, full thickness mice skin, permeation enhancers, transdermal drug delivery

Introduction

Transdermal application is a promising way of drug administration, providing several benefits. Though the outermost layer of skin, the stratum corneum, forms an excellent barrier against permeation of drugs because of its rigid lipid lamellar struc-

* Author for correspondence: E-mail: Roman.Schiller@schering.de

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ture. The most easily permeating drugs are small molecules of moderate lipophilicity. A parabolic dependence is often found between skin permeation and octanol-water partition coefficient P with an optimum value of log~2. At low log P, (i. e. hydrophilic molecules), the permeability is low since partitioning into the skin lipids is low. In this case, the drug permeation can be improved by increase of thermodynamic activity of drug in the transdermal formulation (push), by the use of permeation enhancers (pull), e.g., lauric acid or by physical enhancement. The aim of this study was to improve the skin permeability for highly lipophilic drugs. As model compounds, two new pure antiestrogens (AE 1 – Fig.1a, AE 2 – Fig.1b) were chosen. For this purpose, the effects of several permeation enhancers and various combinations thereof on the diffusion of these drugs through excised skin of hairless mice were determined. Furthermore, the mechanism of permeation enhancement was to be clarified [1]. There are several methods widely used to investigate transdermal permeation enhancement, including microscopy, DSC on stratum corneum, ESR, X-ray diffraction and others. An additional strategy is to determine transdermal fluxes of enhancers and solvents in parallel to the permeant of main interest, thus revealing more insight into the mechanism of permeation enhancement. Although the latter method provides important information with respect to skin permeation, it is used very rarely.

Therefore, in this study the transdermal permeation of propylene glycol (PG), dimethyl isosorbide (DMI), dimethylsulfoxide (DMSO), and lauric acid (LA) from various transdermal fluid formulations were determined in parallel to the permeation of antiestrogen. Thermoanalytic (DSC) measurements of skin lipid phase transition temperatures were performed using full thickness mice skin to investigate the effect of skin lipid softening and the correlation with transdermal delivery of the drug substance.

Materials and methods

Materials

Antiestrogen - AE 1 (11β-fluoro-7α-[5-(methyl-{3-[(4,4,5,5,5-pentafluoropentyl)-sulfanyl]-propyl}-amino)-pentyl]-estra-1,3,5(10)-triene-3,17β-diol), Fig. 1a. Antiestrogen - AE 2 (11β-fluoro-7α-{5-[methyl(7,7,8,8,9,9,10,10,10-nonafluorodecyl)-amino]-pentyl}-estra-1,3,5(10)-triene-3,17β-diol), Fig. 1b, – manufactured by Dr. J. Kroll, Schering AG, Berlin, Germany.

Propylene glycol was purchased from Sigma-Aldrich-Chemie GmbH, Steinheim, Germany. Arlasolve DMI (dimethyl isosorbide) was obtained from ICI surfactants, Middlesbrough, Cleveland. Dimethyl sulfoxide was obtained from Merck KGaA, Darmstadt, Germany. LA, dodecanol, (2-hydroxypropyl)-β-cyclodextrin ($MR \approx 1380$, degree of substitution ≈ 0.6) and benzylpenicilline potassium salt were purchased from Fluka Chemie AG, Buchs, Switzerland. Phosphate buffered saline (PBS) [137 mM sodium chloride and 2.7 mM potassium chloride in

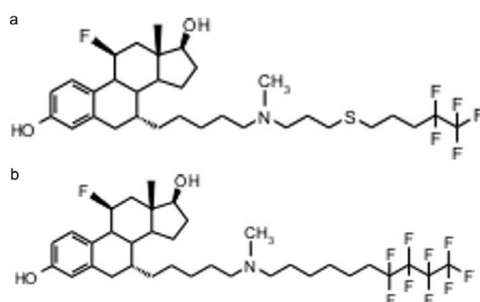

Fig. 1 Chemical structures of AE 1 and AE 2

10 mM phosphate buffer pH = 7.4] was prepared from tablets, purchased from Fluka. Water was double distilled. All other chemicals were of analytical grade.

Drug absorption studies

Sections of full-thickness skin of male hairless mice (MF1 h h^{-1}, supplied by Harlan–Winkelmann, Borchen, Germany) were placed into modified flow-through Franz diffusion cells (diffusion area 2 cm^2, Schering AG, Berlin, Germany). For some studies the stratum corneum was removed from the skin sections by tape-stripping (20×). The donor compartment (stratum corneum side) was filled with a solution of either a single drug or two drugs simultaneously (applied volume: 20 μL, each drug concentration: 2% *w*/*v*). The receptor fluid, consisting of PBS with 3 or 5% *w*/*v* (2-hydroxypropyl)-β-cyclodextrin to provide sink condition and 1 Mio I.U./L benzylpenicilline potassium salt as microbiological stabilizer, was pumped through the diffusion cell by a pneumatic pump (model IPN12/16, Ismatec, Glattbrugg-Zürich, Switzerland) at a flow rate of approximately 1 mL h^{-1}. The receptor fluid was collected in glass vials during a period of 48 h, changing the vials every two hours in the first eight hours of each study and every eight hours thereafter (automatic sample collector, model retriever III/IV, Isco, Lincoln, Nebraska). In case of skin pretreatment the receptor fluid was collected over an additional period of 16 h, changing the vials every four hours. The whole system was maintained at 32°C by a thermostatic water pump (Haake, Karlsruhe, Germany). All acceptor solution fractions were stored at –18°C until HPLC/UV and GC/FID analysis, resp. in order to minimize microbiological contaminations and chemical drug degradation.

For each skin section, cumulated permeated amounts of drugs, enhancers and solvents were plotted *vs.* time. The linear section of each curve was individually extrapolated to determine steady-state flux (slope) and lag-time (point of intersection with time axis). As a third parameter the total amounts permeated during 48 h were calculated.

As typical in studies with biomaterials, data from in-vitro permeation studies have high coefficients of variation. A *t*-test [2] showed that steady-state fluxes differ

significantly ($\alpha \leq 5\%$, $\beta = 10\%$), if they differ at least 2.5-fold, assuming our study design ($n \geq 3$; coefficient of variation $\leq 40\%$).

Assay of estradiol, AE 1 and AE 2

HP Chem Station HPLC 1100 with degasser, low-pressure gradient pump, automatic sampler, diode array detector (Agilent Technologies, Palo Alto, USA), RP 18 Hypersil ODS 3 μm column with a length of 125 mm and 4.6 mm inner diameter (VDS Optilab, Montabaur, Germany) was used for the experiments. The injected volume was 50 μL. Acetonitrile – water – 0.3% (*w*/*v*) trifluoroacetic acid was used for gradient elution with a flow of 1.2 mL min^{-1}.

Assay of PG, DMSO, DMI and LA

HP 5890 Series II gas chromatograph with HP7673A automatic sampler (Agilent Technologies, Palo Alto, USA), and a CPSil 5 CB (Varian Chrompack, Darmstadt, Germany, length: 10 m, internal diameter: 0.53 μm) column was applied. Helium was used as carrier, the temperature of the injector was 280°C. The injected volume was 1 μL. Flame ionization detector was used at FID, 280°C. For the determinations the following temperature program was adjusted: 0–4 min: 70°C, 4.7–9.7 min: 120°C, 10.4–14.4 min: 170°C, 15.9–23.9 min: 270°C, 26.7–30 min: 70°C, heating/cooling rate: 70°C min^{-1}; retention times: 1.4 min (PG), 2.2 min (DMSO), 8.2 min (DMI), 12.6 min (LA).

Aqueous solutions of (2-hydroxypropyl)-β-cyclodextrin contain small amounts of PG, resulting from hydrolytical side chain cleavage. Therefore a blank permeation study was conducted, replacing the skin by a sheet of parafilm. The determined amounts of PG in acceptor fluid fractions from the latter experiment were used as zero values for in-vitro permeation studies.

Partition coefficient measurements

Partition coefficient (*n*-octanol/water) measurements were conducted semi-quantitatively by HPLC retention time determination, according to OECD guideline for testing chemicals 117, using the previously mentioned HPLC-system. RP 18 Hypersil ODS 3 μm column with a length of 125 mm and 4.6 mm internal diameter (VDS Optilab, Montabaur, Germany) was used. The injected volume was 10 μL, furthermore, methanol – 0.02 M aqueous phosphate buffer (pH = 7.0) 75+ 25 was used as eluent (flow rate: 1.0 mL min^{-1}) with addition of various acids (0.01 M) to the aqueous phase. The sample amount for AE 1 was 2, 20 and 200 μg mL^{-1} respectively, dissolved in acetonitrile. All retention time measurements have been done at 22°C.

Differential scanning calorimetry (DSC)

4 cm^2 sections of full-thickness skin of male hairless mice were placed on PBS soaked paper sheets. Various permeation enhancer formulations (40 μL and 13.3 μL respectively) were applied onto the stratum corneum side. 6 h after this treatment, formula-

tions were completely removed from skin surface. Then approximately 10–20 mg of full-thickness skin sections were cut from the treated 4 cm^2 sections and placed in 40 μL aluminium standard pans. Thermoanalytic measurements were conducted using a STARe/Thermal-Analysis-System (Mettler-Toledo, Gießen, Germany, DSC 821/822, Intracooler, Gas-Switch, 200W, FRS-5 sensor). Untreated skin samples as well as pure permeation enhancer formulations were measured as controls. All samples were analysed between –20 to 150°C at 10 K min^{-1} heating rate under nitrogen flow. In a preliminary DSC study, the heating cycle was repeated on the same sample. The second heating curve was analysed, but not significant changes were obtained. Therefore only the results obtained during the first heating runs are reported.

Results and discussion

Passive and unhindered permeation of AE 1

To characterize the passive diffusion of AE 1 through hairless mice skin, 2% solutions of AE 1 in DMI were applied on intact skin of hairless mice. Selected permeation experiments were conducted using estradiol as an internal standard, in order to verify the validity of the permeation experiment. According to Fick's law [3] the simultaneous application of AE 1 and estradiol onto the same skin section resulted in independent diffusion of both drugs. Unhindered diffusion through skin was simulated by removal of stratum corneum (tape-stripping).

Results from all in-vitro permeation studies are summarized in Table 1. Estradiol and AE 1 exhibited passive steady-state fluxes from DMI (formulation A) of approximately 30 ng cm^{-2} h^{-1}. This result is very close to the findings of Günther [2], who obtained a passive diffusion of 55 ng cm^{-2} h^{-1} for estradiol. Unhindered permeation of estradiol and AE 1, respectively through stripped skin (E) was two orders of magnitude higher than the passive permeation (A). These two studies delimit a range between very slow passive diffusion and the unhindered permeation of the lipophilic drug AE 1 through hairless mouse skin. The effectivity of permeation enhancement can be measured by comparison with these values.

Enhancement by single permeation enhancers

PG may be used as solvent for transdermal formulations, but it can also have permeation enhancing properties of its own [4]. For example, the transdermal permeation of estradiol proved to be 10-fold higher from PG solution than from DMI solution (formulation C *vs.* A, Table 1). However, for AE 1 no differences in steady-state fluxes could be detected if PG was used instead of DMI. By the use of dodecanol as a single enhancer (5% *w*/*w*) in a DMI formulation (N), the transdermal permeation of estradiol as well as of AE 1 was improved slightly (2.5-fold). Likewise, the use of LA as a single enhancer (10% *w*/*w*) in a DMI formulation (B) improved transdermal permeation of estradiol as well as AE 1. Enhancement factor for AE 1 was 2, whereas permeation of estradiol was enhanced 10-fold.

Table 1 Permeation of antiestrogens from various fluid formulations (drug concentration 2%; partly with additional 2% content of estradiol as internal standard, passive transdermal diffusion rates of simultaneously applied compounds are independent of each other) through excised skin of hairless mice

Formulation		Compound	*n*	Steady-state flux of AE 1/ ng cm^{-2} h^{-1}	Lag-time/ h	Amount permeated (48 h, 2 cm^2)/μg
Passive Permeation						
A	DMI	AE 1	9	30±16	n. a.	2.5±2.0
		estradiol	6	30±30	n. a.	2.7±1.3
Unhindered permeation through skin without stratum corneum						
E	PG	AE 1	6	6300±3800	3.8±0.6	270±140
		estradiol	3	10200±5100	0	240±90
Formulations containing a single permeation enhancer						
N	DMI,	AE 1	2	80	n. a.	4±3
	5% dodecanol	estradiol	2	80±50	n. a.	6±2
B	DMI,	AE 1	3	70±30	n. a.	8±3
	10% LA	estradiol	3	360±150	n. a.	40±30
C	PG	AE 1	5	50±20	12±5	3±2
		estradiol	6	335±130	11±3	23±8
Formulations containing permeation enhancer combinations						
L	PG, 2% DMSO	AE 1	3	90±18	5±1	6.0±2
M	DMI, 10% LA, and 2% DMSO	AE 1	3	15±1	7.0±2	1±0.5
Formulations containing the unique permeation enhancer combination PG – LA						
G	PG, 10% LA	AE 1	8	5800±1200	11±3	280±45
		AE 2	3	3200±1000	8.0±1.0	190±70
		estradiol	3	7700±1700	0	260±50

PG: propylene glycol, LA: lauric acid; DMI: dimethyl isosorbide; DMSO: dimethyl sulfoxide; n. a.: not available; mean ± standard deviation; *n* = number of determinations.

Enhancement by permeation enhancer combinations

To evaluate potential synergistic effects of permeation enhancer combinations, three different dual enhancer formulations were investigated. The fluid transdermal formulation L (2% DMSO in PG) did not provide significant permeation enhancement for AE 1. Combination of DMSO (2%) and LA (10%) as enhancers was realized in a transdermal fluid formulation M using DMI as solvent. No permeation enhancement for AE 1 was observed from the combination of these enhancers.

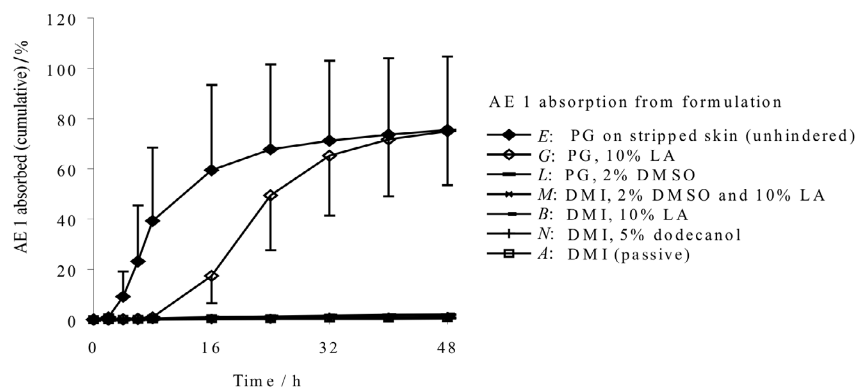

Fig. 2 Cumulated transdermal absorption of the highly lipophilic antiestrogen AE 1 from various fluid formulations through excised skin of hairless mice, in relation to the amounts applied (mean ± standard deviation; n≥3

Finally, a combination of LA (10%) and PG (formulation G) was investigated. In this formulation, PG served as enhancer and as solvent, simultaneously. It turned out, that permeation of AE 1 and estradiol was similar to the unhindered permeation of these drugs through skin without stratum corneum. Thus an extraordinarily high permeation enhancement, namely 100-fold compared to the passive permeation, was observed.

To illustrate these results, AE 1 permeation through excised skin from various fluid formulations is shown in Fig. 2, compared to unhindered permeation through skin without stratum corneum (formulation E). Addition of single enhancers to fluid formulations (B, N *vs.* A) did not increase AE 1 transdermal flux. Similar results were obtained for the enhancer combinations DMSO – PG (L) and DMSO – LA (M), respectively. In contrary, the unique permeation enhancer combination LA – PG (G) significantly increases AE 1 flux to approximately the maximum permeation level through stripped skin. LA and PG are recognized as safe for use in cosmetic products [5, 6]. However, toxicological data for the combination are not available.

Lag-times

It is interesting that steady-state fluxes of the antiestrogens are only obtained after relatively long lag-times of up to 12 h, whereas the onset of the internal standard estradiol permeation was much sooner. This is probably due to accumulation of these highly lipophilic drugs in the stratum corneum lipid matrix. To clarify the reasons for this long lag-time, an in-vitro permeation study was conducted after pretreatment of the skin. For that purpose, three skin sections were pretreated with 20 μL of pure PG for 16 h, after that a 2% solution of AE 1 in formulation G (PG – 10% LA) was applied. Another three skin sections were pretreated for 16 h with formulation G, then a 2% solution of AE 1 in PG was applied. The lag-times and steady-state fluxes of AE 1 are given in Table 2, in comparison to the results of permeation studies without pretreatment.

Table 2 In-vitro permeation study through excised skin of hairless mice: influence of skin pretreatment on the lag-time of AE 1

Pretreatment (16 h)	Permeation study solution of AE 1 (2%) in	Steady-state flux of AE 1/ ng cm^{-2} h^{-1}	Lag time AE 1/ h
–	PG, 10% LA (form. G)	5800±1200	11±3
PG	PG, 10% LA (form. G)	6700±2500	10±4.0
PG, 10% LA (form. G)	PG	6200±800	4.0±0.4
Removal of stratum corneum	PG	6300±3800	4±1

mean ± standard deviation, $n \geq 3$; PG: propylene glycol, LA: lauric acid.

Similar to the findings of Santoyo and Ygartua [7] pretreatment with pure PG did not affect neither lag-time nor steady-state flux of AE 1. Pretreatment with PG – LA provided the same effect as the removal of stratum corneum: the lag-time of AE 1 was reduced to four hours. From these results, it is concluded that the lag-time for AE 1 comprises a time period of approximately 6 h which is necessary for the response of the enhancer combination and a further time period of approximately 4 h which represents the duration of AE 1 permeation through the hydrophilic skin layers.

Permeation study with AE 2

The outstanding enhancing effect of this transdermal fluid formulation (G) (PG – LA 9 + 1) for highly lipophilic drugs was also verified for another drug AE 2, which is even more lipophilic, exhibiting the extremely high octanol-water partition coefficient of $\log P = 7.8$. In addition to its high lipophilicity AE 2 exhibited a very low solubility in PG, so the 2% fluid formulation was not a solution, but a suspension. The passive permeation from this suspension was below limit of determination. However, the steady-state fluxes of AE 2 from formulation G were in the same order of magnitude as those of AE 1, reduced by not more than a factor of 2 (Table 1). Thus, this permeation enhancer combination is able to enhance transdermal permeation of the investigated highly lipophilic drugs markedly. This permeation enhancer system could also be used for other lipophilic drugs. The investigation on that matter is beyond the scope of this article.

Transdermal fluxes of enhancers and solvents

Results from in-vitro permeation studies of enhancers and solvents are summarized in Table 3. The diffusion of several compounds simultaneously applied onto the same skin section are independent of each other, as long as none of this compound affects the skin barrier function [3]. Thus, a change in the permeation rate of one single permeant by addition of a second compound indicates an influence of this second compound on the skin barrier properties. On the other hand, interdependences of compound permeation indicate synergistic effects on skin permeability.

Table 3 Influence of fluid formulation composition on permeation of enhancers and solvents through excised skin of hairless mice

Formulations				In-vitro permeation			
Excipient		Parts	Dose*/ mg	Total*/ mg	Steady-state flux/ $\mu g\ cm^{-2}\ h^{-1}$	Lag-time/ h	Amount permeated/ μg (16 h, 2 cm^2)
Passive Permeation							
A	DMI	100	16.5	16.5	193	1.2	18400
B	LA	10	1.6	16.5	4	3.6	430
	DMI	90	14.9		212	0.7	1 4700
C	PG	100	16.5	16.5	63±21	3.1±1.2	5900±1800
Formulations on skin without stratum corneum (unhindered permeation)							
D	DMI	100	16.5	16.5	2760±120	0	23000±1500
E	PG	100	16.5	16.5	1700±420	0	18000±2800
F	LA	10	1.6	16.5	46±19	2.0±0.7	1800±200
	PG	90	14.9		1870±200	0	17000±1400
Formulations containing the unique permeation enhancer combination PG – LA							
G	LA	10	1.6	16.5	44±20	2.9±0.9	1300±700
	PG	90	14.9		2020±437	0	17000±3000
H	LA	3.3	0.5	15.4	16±1	4.8±2.0	350±50
	PG	90	14.9		1180±63	3.1±1.3	12000±2100
I	LA	3.3	0.5	5.5	14±4	1.6±0.7	340±110
	PG	30	5.0		646±43	0	5400±500
J	LA	10	1.6	16.5	45±44	4.4±0.6	1000±1000
	PG	30	5.0		75±90	0.8±1.4	1600±2000
	DMI	60	9.9		400±293	2.1±1.9	8600±3400
K	LA	3.3	0.5	16.5	2±2	5.9±1.3	50±40
	PG	30	5.0		24±9	0	810±230
	DMI	66.7	11.0		122±111	1.9±1.5	3600±2600

Table 3 Continued

Formulations				In-vitro permeation			
Excipient		Parts	Dose*/ mg	Total*/ mg	Steady-state flux/ μg cm^{-2} h^{-1}	Lag-time/ h	Amount permeated/ μg (16 h, 2 cm^2)
Formulations containing other permeation enhancer combinations							
L	DMSO	2	0.4	20.0	4±1	3.2±0.5	180±30
	PG	98	19.6		83±13	2.4±0.2	4600±1000
M	DMSO	2	0.4	20.0	8±2	1.0±0.6	160±10
	LA	10	2.0		19±4	3.3±0.3	1800±300
	DMI	88	17.6		342±90	1.2±0.1	16300±860

LA: lauric acid; PG: propylene glycol; DMI: dimethyl isosorbide; DMSO: dimethyl sulfoxide; *dose of single compound and total dose of formulation, resp. per 2 cm^2 diffusion area; A, B: n=1; C – M: mean ± standard deviation, n=2 (M), n=3 (D, F, H – L), n=6 (C, E) and n=21 (G), resp.

Passive transdermal diffusion of PG, LA and DMI were found to be approximately 60, 4 and 200 μg cm^{-2} h^{-1}, respectively (formulations C, B and A, respectively). Unhindered permeation through stripped skin was more than 10-fold higher in all cases (E, F and D, respectively).

The transdermal fluid formulation G (PG – LA 9 + 1) leads to an extraordinarily high permeation enhancement of highly lipophilic drugs. Furthermore, it turned out

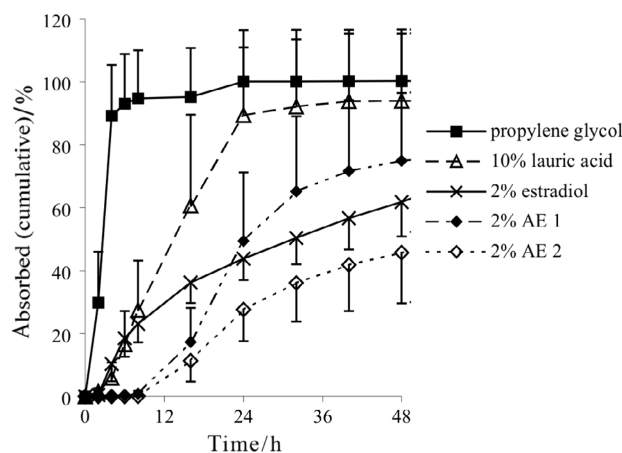

Fig. 3 Cumulated transdermal absorption of all excipients of transdermal fluid formulation G, based on the unique permeation enhancer formulation propylene glycol – lauric acid (9+1) through excised skin of hairless mice, in relation to the amounts applied (mean ± standard deviation; AE 8: n =1; AE 2, estradiol: n =3; LA: n =24; PG: n =21; drug concentrations 2%)

that this formulation also achieves high steady-state fluxes of excipients (Fig. 3), namely 44 $\mu g\ cm^{-2}\ h^{-1}$ LA and 2020 $\mu g\ cm^{-2}\ h^{-1}$ PG. These values are similar to the maximal permeation rates of these compounds through skin without stratum corneum (E and F). Furthermore, they prove a mutual and synergistic permeation enhancement of PG and LA. The high transdermal LA fluxes in the aforementioned experiments are contradictory to the findings of Komata *et al*. [8], who applied a formulation containing PG and LA, onto skin and did not find LA in acceptor fluid. In consequence they postulated a LA accumulation in stratum corneum. However, since an aqueous acceptor medium (PBS, pH = 7.3) without addition of any solubilizer was used, the too low solubility of LA hampered the experiment.

Effect of PG and LA concentrations

To investigate the effects of dose and concentration of PG and LA onto the skin barrier function, the composition of the permeation enhancing formulation PG – LA was varied: Reduction of LA concentration to one third (formulation H) also reduced significantly LA steady-state flux to one third, whereas permeation of PG was slightly slowed down. However, if the ratio PG – LA was kept constant, but the total amount of formulation was reduced to one third (I), steady-state fluxes of LA and PG as well were significantly reduced to one third, whereas lag-times were not affected. To reduce the concentration of PG, which is needed for practical reasons also as a solvent for the solid LA in the described formulations, this compound was partly replaced by DMI, assuming that DMI acts as an indifferent solvent. Reduction of PG concentration to one third (J) caused a steady-state flux of PG of 75 $\mu g\ cm^{-2}\ h^{-1}$, nearly equivalent to passive permeation of PG (C), i. e. only 5% of the flux from formulation G. However, permeation of LA is not affected. If both concentrations of PG and of LA are reduced to one third (K), also LA steady-state flux is significantly reduced (only 5% compared to G) to the passive permeation level (B), too. In both cases, permeation of DMI is not significantly different from passive transdermal diffusion (A). However, DMI steady-state flux was enhanced 3-fold, if LA concentration was increased by the factor 3 (J *vs.* K). It is noteworthy, that formulations I and K contained identical enhancer amounts per skin area, but resulted in significantly different transdermal fluxes. The reduction of enhancer concentration via dilution with the inert solvent DMI led to a marked deterioration of transdermal permeation of both enhancers.

Transdermal formulations containing other combinations of permeation enhancers were investigated, too: DMSO – PG and DMSO – LA. The latter combination was realized by using DMI as an indifferent solvent. Addition of 2% *w*/*w* DMSO to PG (L) did not affect the transdermal permeation of PG (C). DMSO steady-state flux from this formulation was 3.5 $\mu g\ cm^{-2}\ h^{-1}$. Using the permeation enhancer combination DMSO – LA (M), only a slight mutual permeation enhancement was observed. However, this did not cause any permeation enhancement for DMI (compared to B) or the highly lipophilic antiestrogen AE-1 (Table 1).

These results indicate a mutual and synergistic permeation enhancement of PG und LA. However, synergism is only observed, if both enhancers are sufficiently concen-

trated in transdermal fluid formulations. This is in good correspondence to the findings of Oh *et al*. [9], who reported a moderate effect of a formulation containing 5% lauric acid. Total amount of enhancer (per skin area) does not seem to be the crucial parameter.

It was concluded that the permeation enhancement of LA must be due to effects onto the skin. Tanojo [10], Barry [11] and Suhonen [12] already described lipid-fluidising effects of fatty acids by insertion into the intercellular lipid matrix. In addition to this Cumming and Winfield [13] showed the lipid-fluidising effect of LA sodium salt when applying a DSC method.

In order to investigate further the potential fluidising effect of LA onto stratum corneum lipids, a DSC study with full skin from hairless mice after treatment with various fluid formulations was carried out. Results of this study are shown in Fig. 4. Untreated skin samples showed three endothermic peaks at 33, 56 and 62°C, the latter two being attributed to phase transitions of the stratum corneum lipids, according to the results of Leopold and Lippold [14], who investigated human stratum corneum.

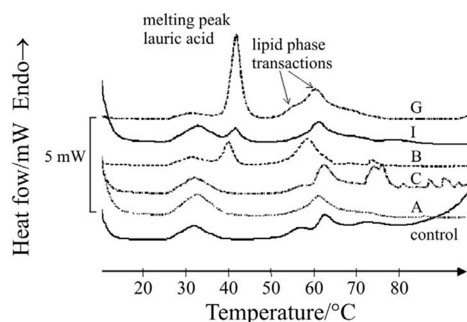

Fig. 4 DSC curves of excised full skin samples of hairless mice after treatment with various fluid permeation enhancer formulations for 6 h (compositions are given in Table 3; control untreated skin sample)

Skin treatment with pure solvents, PG (formulation C) and DMI (A), respectively slightly altered the phase transition temperatures, as shown in Table 4. However, formulations containing LA (esp. B and G) markedly decreased these temperatures, indicating a lipid-fluidising action of LA. In those cases an additional endothermic peak was observed at 39–41°C, which is attributed to the melting of LA. Since the DSC program started at –20°C, it is very likely that LA in the skin samples crystallized. Therefore, the enthalpy of the peak at 39–41°C represents the content of crystalline LA in the skin sample. It is noteworthy, that formulation G (PG – LA) leads to significantly higher deposition of LA into the skin, compared to formulation B (DMI – LA). These findings correspond to the results from our in-vitro permeation studies, where a significantly higher transdermal steady-state flux of LA from formulation G in comparison to formulation B was observed (Table 1). From these results, a lipid-fluidising effect of LA is concluded.

Table 4 Lipid phase transition temperatures and LA melting peaks of full skin samples of hairless mice, treated for 6 h with various fluid permeation enhancer formulations, determined by DSC

Skin treatment			DSC results			
	Formulation	Applied volume/ mL 4 cm^{-2}	Melting peak LA*/ °C	Melting enthalpy LA/ J g^{-1}	Skin lipid phase trans. T/°C peak 1	peak 2
	control^0	–	–	–	56.0±0.1	62.0±0.3
A	DMI	40	–	–	55.5±0.9	61±1
C	PG	40	–	–	56.3±0.3	62±1
B	DMI – LA	40	39.7±0.1	1.6±0.6	53.2±0.1	58±0.5
I	PG – LA	16.6	40.9±0.4	0.8±0.7	56.2±0.4	61±0.4
G	PG – LA	40	41.4±0.1	5.3±1.4	54.9±0.2	60.0±0.3

mean ± standard deviation, n=5; DMI: dimethyl isosorbide, PG: propylene glycol; LA: lauric acid; control^0: untreated skin sample; *melting peak (pure LA): 43.6±0.14°C

However, LA did not increase the fluxes of the highly lipophilic antiestrogen, when it was applied in the inert solvent DMI. Despite the fact, that neither passive diffusion of the tested antiestrogen nor its unhindered permeation through stripped skin was influenced by the choice of solvent (DMI or PG), the permeation enhancing effect of LA was only observed, when PG was used as solvent. From these findings it is concluded, that the site of action of both PG and LA is the intercellular lipid matrix of stratum corneum. Obviously, PG increases the solubility of LA in the stratum corneum and in consequence LA partition into this outermost skin layer.

On the other hand, it had been shown previously by Takeuchi *et al*. [9], that fatty acids increase PG penetration into skin, too. Summarizing these findings, the mutual permeation enhancement of PG and LA is due firstly to the enhanced solubility of LA in and its penetration into the stratum corneum, both being caused by PG. Secondly it is due to enhanced PG permeability, which is caused by LA insertion between the intercellular lipids of the stratum corneum. Therefore, in this enhancer combination LA acts as a 'co-enhancer' and PG as a 'co-solvent', according to the terminology introduced by Barry [11].

By this unique combination the barrier is modified in a way which allows the highly lipophilic antiestrogens to permeate easily through the skin. So, from this formulation AE 1 steady-state fluxes of 5.8 μg cm^{-2} h^{-1} were observed, thus representing approximately the same value compared to the unhindered permeation through skin without stratum corneum.

Conclusions

In conclusion, it was shown, that an outstanding permeation enhancement can be achieved for highly lipophilic drugs by the use of the enhancer combination propylene glycol – lauric acid. Furthermore, it was demonstrated that this is due to mutual

permeation enhancement of these two enhancers and their synergistic lipid-fluidising action in stratum corneum. The grade of temperature shift on the skin lipids to lower temperatures can be correlated with softening effects and the enhancement potential of the formulation. Based on these results, a fast screening tool for enhancer formulations was achieved, using DSC measurements.

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