Transdermal Delivery of Highly Lipophilic Drugs: *In Vitro* Fluxes of Antiestrogens, Permeation Enhancers, and Solvents from Liquid Formulations

Adrian P. Funke,[1] Roman Schiller,[1] Hans W. Motzkus,[1] Clemens Günther,[2] Rainer H. Müller,[3] and Ralph Lipp[1,4]

Received January 21, 2002; accepted February 4, 2002

Purpose. Highly lipophilic basic drugs, the antiestrogens AE 1 (log P = 5.82) and AE 2 (log P = 7.8) shall be delivered transdermally.
Methods. Transdermal permeation of drugs, enhancers, and solvents from various fluid formulations were characterized by *in-vitro* permeation studies through excised skin of hairless mice. Furthermore, differential scanning calorimetry (DSC) measurements of skin lipid phase transition temperatures were conducted.
Results. Transdermal flux of highly lipophilic drugs was extraordinarily enhanced by the unique permeation enhancer combination propylene glycol–lauric acid (9 + 1): steady-state fluxes of AE 1 and AE 2 were as high as 5.8 $\mu g \cdot cm^{-2} \cdot h^{-1}$ and 3.2 $\mu g \cdot cm^{-2} \cdot h^{-1}$, respectively. This dual enhancer formulation also resulted in a marked increase in the transdermal fluxes of the enhancers. Furthermore, skin lipid phase transition temperatures were significantly reduced by treatment with this formulation.
Conclusion. Transdermal delivery of highly lipophilic drugs can be realized by using the permeation enhancer combination propylene glycol–lauric acid. The extraordinary permeation enhancement for highly lipophilic drugs by this formulation is due to mutual permeation enhancement of these two enhancers and their synergistic lipid-fluidising activity in the stratum corneum.

KEY WORDS: transdermal drug delivery; permeation enhancers; propylene glycol; lauric acid; highly lipophilic drug; antiestrogens; differential scanning calorimetry (DSC).

INTRODUCTION

Transdermal application is a promising way of drug administration, providing several benefits: for example a potential hepatic first pass effect can be avoided, and constant drug delivery during a long period up to one week is feasible (1–4). However, the outermost layer of skin, the stratum corneum forms an excellent barrier against permeation of drugs, because of its rigid lipid lamellar structure. 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 P ≈ 2 (5,6). 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 (7) by increase of thermodynamic activity of drug in the transdermal formulation (push), by the use of permeation enhancers (pull), e.g., lauric acid (8,9) or by physical enhancement strategies, such as iontophoresis (10) or sonophoresis (11). But also at high log P values, (i.e., highly lipophilic molecules), the permeability is low. This is probably due to accumulation of lipophilic drugs in stratum corneum because of low aqueous solubility. Whereas there are many examples given in the literature with respect to permeation enhancement of hydrophilic and moderately lipophilic drugs (4,5), not much has been reported in the area of permeation enhancement of highly lipophilic drugs so far.

The aim of this study was to improve the skin permeability for highly lipophilic drugs. As model compounds two new pure antiestrogens were chosen, AE 1 (log P = 5.82, determined at pH = 7.0) and AE 2 (log P = 7.8). Chemical structures are given in Fig. 1. For that purpose, the effects of several permeation enhancers and various combinations thereof on the diffusion of these drugs through excised skin of hairless mice were to be determined.

Furthermore, the mechanism of permeation enhancement was to be clarified. There are several methods widely used to investigate transdermal permeation enhancement, including microscopy (12), DSC (13,14), ESR (15), X-ray diffraction (16) 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. Among the reports on parallel investigation of drug and enhancer permeation, there are so far no studies which have consequently evaluated the skin permeation of all relevant excipient besides the drug of interest. For example Wotton *et al.* (17) studied the transdermal permeation of metronidazole and also evaluated propylene glycol permeation, but not that of the simultaneously applied permeation enhancer azone.

Therefore, in this study transdermal permeation of propylene glycol (PG), dimethyl isosorbide (DMI), dimethyl sulfoxide (DMSO) and lauric acid (LA) from various transdermal fluid formulations shall be determined in parallel to the permeation of the antiestrogens. In addition, thermoanalytic (DSC) measurements of skin lipid phase transition temperatures shall be performed, to further increase the insight into the mechanism of permeation enhancement.

MATERIALS AND METHODS

Materials

Estradiol, 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) and 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) were 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,

[1] Pharmaceutical Development, Schering AG, 13342 Berlin, Germany.
[2] Preclinical Pharmacokinetics, Schering AG, 13342 Berlin, Germany.
[3] Department of Pharmaceutics, Biopharmaceutics and Biotechnology, Freie Universität Berlin, Kelchstr. 31, 12169 Berlin, Germany.
[4] To whom correspondence should be addressed. (e-mail: ralph.lipp@schering.de).

0724-8741/02/0500-0661/0 © 2002 Plenum Publishing Corporation

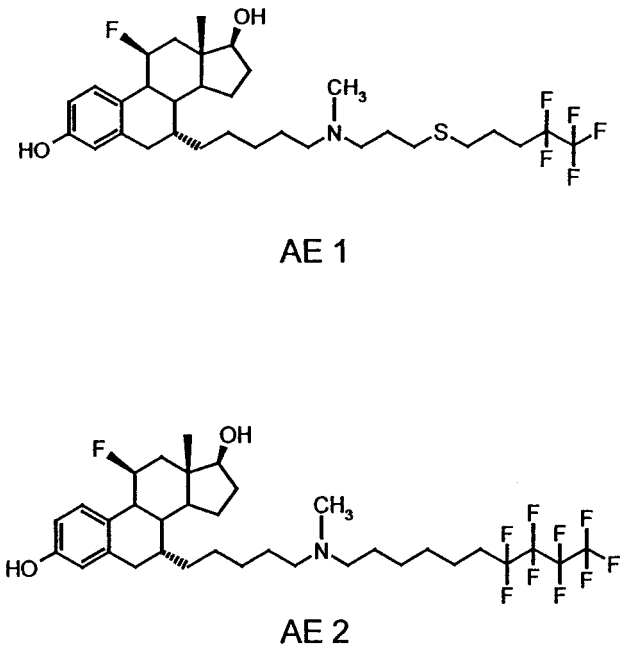

Fig. 1. Chemical structures of AE 1 and AE 2.

(Middlesbrough, Cleveland). Dimethyl sulfoxide was obtained from Merck KGaA, (Darmstadt, Germany). LA, dodecanol, (2-hydroxypropyl)-β-cyclodextrin (MR ≈ 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 10 mM phosphate buffer pH = 7.4) was prepared from tablets, purchased from Fluka. Water was double destilled. All other chemicals were of analytical grade.

Drug Absorption Studies

Sections of full-thickness skin of male hairless mice (MF1hr/hr, 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. The receptor fluid was collected in glass vials during a period of 48 h, changing the vials every 2 h in the first 8 h of each study and every 8 h 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 4 h. 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, respectively, to minimize microbiological contaminations and chemical drug degradation.

For each skin section, cumulated permeated amounts of drugs, enhancers and solvents were plotted against 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 variance. A *t* test (18) 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 variance ≤ 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, California), column: RP 18 Hypersil ODS 3 μm, length 125 mm, inner diameter 4.6 mm (VDS optilab, Montabaur, Germany), injection volume 50 μL, eluent: gradient acetonitrile–water–0.3 % (w/v) trifluoroacetic acid, flow 1.2 mL/min, detection: UV 230 nm, external standards.

Assay of PG, DMSO, DMI, and LA

HP 5890 Series II gas chromatograph with HP7673A automatic sampler (Agilent Technologies, Palo Alto, California), column: CPSil 5 CB (Varian Chrompack, Darmstadt, Germany), length: 10 m, ID: 0.53 μm, carrier: helium, injector: 280°C, 1 μL, split injection (0.5 min splitless), detector: FID, 280°C, external standard; oven temperature program: 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; 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 aforementioned HPLC-system; column: RP 18 Hypersil ODS 3 μm, length 125 mm, inner diameter 4.6 mm (VDS optilab, Montabaur, Germany), injection volume 10 μL, eluent: methanol–0.02 M aqueous phosphate buffer (pH = 7.0) 75+ 25 with additions of various acids (0.01 M) to the aqueous phase, flow 1.0 mL/min, detection: UV 230 nm, samples: 2, 20, and 200 μg/mL AE 1, respectively, dissolved in acetonitrile. All retention time measurements were conducted at 22°C.

Differential Scanning Calorimetry (DSC)

Sections (4 cm^2) sections of full-thickness skin of male hairless mice were placed on PBS soaked paper sheets. Vari-

ous permeation enhancer formulations (40 μL and 13.3 μL, respectively), were applied onto the stratum corneum side. Six hours after this treatment, formulations were completely removed from skin surface. Then, full-thickness skin sections of approximately 10–20 mg weight were cut from these treated 4 cm^2 sections and placed in 40 μL aluminium standard pans. Thermoanalytic measurements were conducted using a Thermoanalysesystem TA 8000 (Mettler-Toledo, Gießen, Germany, DSC Meβzelle 8212, Intracooler, Gasbox 200W). Untreated skin samples as well as pure permeation enhancer formulations served as control. All samples were analyzed over a heating range from –20°C to 150°C at 10 K/min under nitrogen. In a preliminary DSC study, the heating cycle was repeated on the same sample. The second heating curve was analyzed, but did not show any significant changes. Therefore only the results of the first heating 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, to verify the validity of the permeation experiment. According to Fick's law (7) 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 I. Estradiol and AE 1 exhibited passive steady-state fluxes from DMI (formulation **A**) of approximately 30 $ng \cdot cm^{-2} \cdot h^{-1}$. This result is very close to the findings of Günther (18), who obtained a passive diffusion of 55 $ng \cdot cm^{-2} \cdot h^{-1}$ 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 (19). For example, the transdermal permeation of estradiol proved to be10-fold higher from PG solution than from DMI solution (formulation **C** vs. **A**, see Table I). 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.

Enhancement by Permeation Enhancer Combinations

To evaluate potential synergistic effects of permeation enhancer combinations, three different dual enhancer formu-

TABLE I. Permeation of Antiestrogens from Various Fluid Formulations through Excised Skin of Hairless Mice

	Formulation	Compound	n	Steady-state flux of AE 1 $ng \cdot cm^{-2} \cdot h^{-1}$	Lag-time h	Amount permeated (48 h, 2 cm^2) μg
Passive Permeation						
A	DMI	AE 1	9	29 ± 16	n. a.	2.5 ± 2.0
		estradiol	6	33 ± 29	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	236 ± 85
Formulations containing a single permeation enhancer						
N	DMI, 5% dodecanol	AE 1	2	77	n. a.	4.2 ± 3.2
		estradiol	2	78 ± 54	n. a.	6.4 ± 2.0
B	DMI, 10% LA	AE 1	3	67 ± 28	n. a.	7.5 ± 2.5
		estradiol	3	362 ± 154	n. a.	41 ± 30
C	PG	AE 1	5	49 ± 20	12 ± 5	3.4 ± 2.1
		estradiol	6	335 ± 131	11 ± 3	23 ± 8
Formulations containing permeation enhancer combinations						
L	PG, 2% DMSO	AE 1	3	90 ± 18	4.6 ± 0.9	6.0 ± 2.2
M	DMI, 10% LA, and 2% DMSO	AE 1	3	15 ± 1	7.0 ± 2.3	1.1 ± 0.5
Formulations containing the unique permeation enhancer combination PG–LA						
G	PG, 10% LA	AE 1	8	5800 ± 1200	10.7 ± 3.3	280 ± 45
		AE 2	3	3200 ± 1000	8.0 ± 1.0	190 ± 67
		estradiol	3	7700 ± 1700	0	257 ± 45

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

Note: 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.

lations 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.

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 a several 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 (20,21). However, toxicological data for the combination are not available.

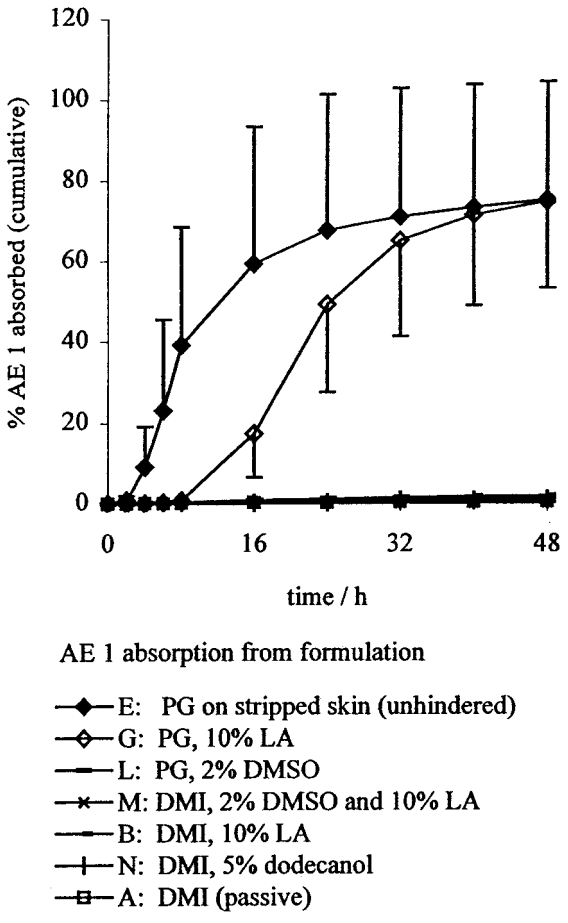

Fig. 2. Cumulated transdermal absorption of the highly lipophilic antiestrogen AE 1 from various fluid formulations through excised skin of hairless mice, in relation to the amounts applied (mean ± standard deviation; n ≥ 3).

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, 3 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 3 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 II, in comparison to the results of permeation studies without pretreatment.

Similar to the findings of Santoyo and Ygartua (22), 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 4 h. 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

TABLE II. *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	10.7 ± 3.3
PG	PG, 10% LA (form. G)	6700 ± 2500	9.8 ± 4.0
PG, 10% LA (form. G)	PG	6200 ± 800	4.0 ± 0.4
Removal of stratum corneum	PG	6300 ± 3800	3.8 ± 0.6

Note: mean ± standard deviation, n ≥ 3; PG: propylene glycol, LA: lauric acid.

of AE 1, reduced by not more than a factor 2 (see Table I). 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, such as fentanyl. This investigation 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 III. The diffusion of several compounds simultaneously applied onto the same skin section are independent of each other, as long as none of this compounds affects the skin barrier function (7). 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.

Passive transdermal diffusion of PG, LA and DMI were found to be approximately 60 $\mu g \cdot cm^{-2} \cdot h^{-1}$, 4 $\mu g \cdot cm^{-2} \cdot h^{-1}$ and 200 $\mu g \cdot cm^{-2} \cdot h^{-1}$, 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 PG–LA 9 + 1 (**G**) leads to an extraordinarily high permeation enhancement of highly lipophilic drugs. Furthermore, it turned out that this formulation also achieves high steady-state fluxes of excipients (Fig. 3), namely 44 $\mu g \cdot cm^{-2} \cdot h^{-1}$ LA and 2020 $\mu g \cdot cm^{-2} \cdot h^{-1}$ PG. These values are similar to the maximal permeation rates of these compounds through skin without stratum corneum (see **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.* (23), 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,

TABLE III. Influence of Fluid Formulation Composition on Permeation of Enhancers and Solvents through Excised Skin of Hairless Mice

					In Vitro Permeation		
Formulation	Excipient	Parts	Dose* mg	Total* mg	Steady-state flux $\mu g \cdot cm^{-2} \cdot h^{-1}$	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	425
	DMI	90	14.9		212	0.7	14700
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	22960 ± 1500
E	PG	100	16.5	16.5	1700 ± 420	0	18000 ± 2770
F	LA	10	1.6	16.5	46 ± 19	2.0 ± 0.7	1773 ± 229
	PG	90	14.9		1870 ± 200	0	16790 ± 1370
Formulations containing the unique permeation enhancer combination PG–LA							
G	LA	10	1.6	16.5	44 ± 20	2.9 ± 0.9	1320 ± 695
	PG	90	14.9		2020 ± 437	0	16900 ± 3080
H	LA	3.3	0.5	15.4	16 ± 1	4.8 ± 2.0	347 ± 52
	PG	90	14.9		1180 ± 63	3.1 ± 1.3	12050 ± 2130
I	LA	3.3	0.5	5.5	14 ± 4	1.6 ± 0.7	336 ± 114
	PG	30	5.0		646 ± 43	0	5440 ± 510
J	LA	10	1.6	16.5	45 ± 44	4.4 ± 0.6	1050 ± 970
	PG	30	5.0		75 ± 90	0.8 ± 1.4	1580 ± 1950
	DMI	60	9.9		400 ± 293	2.1 ± 1.9	8620 ± 3390
K	LA	3.3	0.5	16.5	2 ± 2	5.9 ± 1.3	50 ± 42
	PG	30	5.0		24 ± 9	0	817 ± 232
	DMI	66.7	11.0		122 ± 111	1.9 ± 1.5	3580 ± 2630
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 ± 230
	DMI	88	17.6		342 ± 90	1.2 ± 0.1	16300 ± 856

Note: 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.

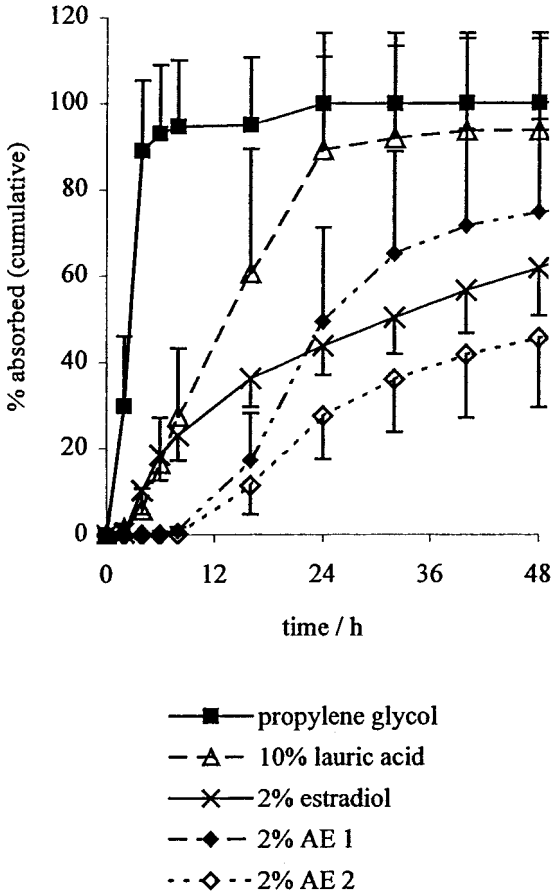

Fig. 3. Cumulated transdermal absorption of all excipients of transdermal fluid formulation G, based on the unique permeation enhancer formulation propylene glycol – lauric acid (9+1) through excised skin of hairless mice, in relation to the amounts applied (mean ± standard deviation; AE 1: n = 8; AE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

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 $\mu g \cdot cm^{-2} \cdot h^{-1}$, 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 $\mu g \cdot cm^{-2} \cdot h^{-1}$. 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 (see Table I).

These results indicate a mutual and synergistic permeation enhancement of PG und LA. However, synergism is only observed, if both enhancers are sufficiently concentrated in transdermal fluid formulations. This is in good correspondence to the findings of Oh *et al.* (24), 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.

Mechanistical Considerations

A possible mechanism of permeation enhancement by fatty acids is the formation of ion pairs with basic drugs, e.g., propranolol (25). To elucidate whether permeation enhancement for AE 1 by LA is due to an ion pair formation of these compounds, semi-quantitative HPLC measurements of partition coefficient were conducted, combining antiestrogen AE 1 (injected as acetonitrile solution) with a broad variety of acids, including phosphoric acid, acetic acid, tartaric acid, trifluoroacetic acid, D-glucuronic acid, 3-methyl butyric acid, lactic acid, glycolic acid and LA (added to the eluent). Retention times of AE 1 should be dependent on the pH-value of the eluents investigated, but in case of ion pair formation diverging lipophilicity should be expected. However, retention time of AE 1 was strongly dependent on the pH-value of the eluent (26). From this data it was concluded that AE 1 does not form ion pairs, neither with LA nor with the other acids tested.

Thermoanalytical Studies

Furthermore, it was concluded that the permeation enhancement of LA must be due to effects onto the skin. Barry (27) and Suhonen (28) already described lipid-fluidising effects of fatty acids by insertion into the intercellular lipid matrix. In addition to this Cumming and Winfield (14) showed the lipid-fluidising effect of LA sodium salt when applying a DSC method.

To further investigate 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°C, 56°C, and 62°C, the latter two being attributed to phase transitions of the stratum corneum lipids, according to the results of Leopold and Lippold (29), who investigated human stratum corneum.

Skin treatment with pure solvents, PG (formulation **C**) and DMI (**A**), respectively, slightly altered the phase transition temperatures, as shown in Table IV. However, formulations containing LA (especially **B** and **G**) markedly decreased

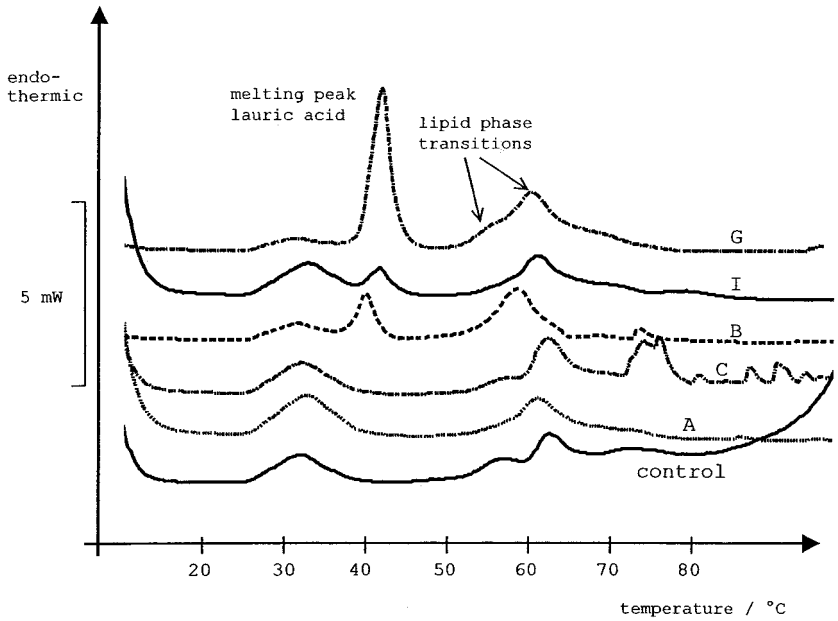

Fig. 4. Differential scanning calorimetry plots of excised full skin samples of hairless mice after treatment with various fluid permeation enhancer formulations for 6 h (compositions are given in Table III; control: untreated skin sample).

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 I). From these results, a lipid-fluidising effect of LA is concluded.

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 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 its 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.* (30), 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

TABLE IV. 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 Differential Scanning Calorimetry (DSC)

			DSC results			
					Skin lipid phase transition temperatures	
Skin treatment	Formulation	Applied volume μL/4 cm²	Melting peak lauric acid* °C	Melting enthalpy lauric acid J/g	Peak 1 °C	Peak 2 °C
	Control[0]	—	—	—	56.0 ± 0.1	62.0 ± 0.3
A	DMI	40	—	—	55.5 ± 0.9	60.6 ± 0.9
C	PG	40	—	—	56.3 ± 0.3	61.9 ± 0.7
B	DMI–LA	40	39.7 ± 0.1	1.6 ± 0.6	53.2 ± 0.1	58.4 ± 0.5
I	PG–LA	16.6	40.9 ± 0.4	0.8 ± 0.7	56.2 ± 0.4	60.6 ± 0.4
G	PG–LA	40	41.4 ± 0.1	5.3 ± 1.4	54.9 ± 0.2	60.0 ± 0.3

Note: Mean ± standard deviation, n = 5; DMI: dimethyl isosorbide, PG: propylene glycol; LA: lauric acid;
[0] control: untreated skin sample;
*Melting peak (pure lauric acid): 43.6 ± 0.14°C.

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 (27).

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 $\mu g \cdot cm^{-2} \cdot h^{-1}$ were observed, thus representing approximately the same value compared to the unhindered permeation through skin without stratum corneum.

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.

REFERENCES

1. B. W. Barry. *Dermatological formulations: Percutaneous absorption*, Marcel Dekker, New York, 1983.
2. Y. W. Chien. Advances in transdermal systemic medication. In Y. W. Chien (ed.), *Transdermal controlled systemic medications*, Marcel Dekker, New York, 1987 pp.1–24.
3. M. Dittgen. Transdermale Therapeutische Systeme. In R. H. Müller and G. E. Hildebrand (eds.), *Pharmazeutische Technologie: Moderne Arzneiformen*, Wiss. Verl. Ges., Stuttgart, 1997 pp. 81–104.
4. H. Schaefer and T. E. Redelmeier. *Skin Barrier: Principles of percutaneous absorption*, Karger, Basel, 1996.
5. R. S. Hinz, C. R. Lorence, C. D. Hodson, C. Hansch, L. L. Hall, and R. H. Guy. Percutaneous penetration of para-substituted phenols *in vitro. Fundam. Appl. Toxicol.* **17**:575–583 (1991).
6. J. Hadgraft. Dermal and transdermal drug design. *Int. J. Pharm. Med.* **13**:155–158 (1999).
7. A. Pardo, Y. Shiri, and S. Cohen. Percutaneous absorption of physostigmine: optimization of delivery from a binary solvent by thermodynamic control. *J. Pharm. Sci.* **79**:573–578 (1990).
8. T. Ogiso and M. Shintani. Mechanism for the enhancement effect of fatty acids on the percutaneous absorption of propranolol. *J. Pharm. Sci.* **79**:1065–1071 (1990).
9. B. J. Aungst, J. A. Blake, and M. A. Hussain. Contributions of drug solubilization, partitioning, barrier disruption, and solvent permeation to the enhancement of skin permeation of various compounds with fatty acids and amines. *Pharm. Res.* **7**:712–718 (1990).
10. J. Hadgraft. Recent developments in topical and transdermal delivery. *Eur. J. Drug. Metab. Pharmacokinet.* **21**:165–173 (1996).
11. S. Mitragotri, D. A. Edwards, D. Blankschtein, and R. Langer. A mechanistic study of ultrasonically-enhanced transdermal drug delivery. *J. Pharm. Sci.* **84**:697–706 (1995).
12. S. J. Jiang, Y. K. Kim, and S. H. Lee. The ultrastructural changes of stratum corneum lipids after application of oleic acid in propylene glycol. *Ann. Dermatol.* **10**:153–158 (1994).
13. H. Tanojo, J. A. Bouwstra, H. E. Junginger, and H. E. Boddé. *In vitro* human skin barrier modulation by fatty acids: skin permeation and thermal analysis studies. *Pharm. Res.* **14**:42–49 (1997).
14. K. I. Cumming and A. J. Winfield. *In vitro* evaluation of a series of sodium carboxylates as dermal penetration enhancers. *Int. J. Pharm.* **108**:141–148 (1994).
15. S. Kitagawa, A. Hosokai, Y. Kaseda, N. Yamamoto, Y. Kaneko, and E. Matsuoka. Permeability of benzoic acid derivatives in excised guinea pig dorsal skin and effects of L-menthol. *Int. J. Pharm.* **161**:115–122 (1998).
16. P. A. Cornwell, B. W. Barry, J. A. Bouwstra, and G. S. Gooris. Modes of action of terpene penetration enhancers in human skin; differential scanning calorimetry, small-angle X-ray diffraction and enhancer uptake studies. *Int. J. Pharm.* **127**:9–26 (1996).
17. P. K. Wotton, B. Mollgaard, J. Hadgraft, and A. Hoelgaard. Vehicle effect on topical drug delivery; III: Effect of azone on the cutaneous permeation of metronidazole and propylene glycol. *Int. J. Pharm.* **24**:19–26 (1985).
18. C. Günther. *In vitro- und in vivo-Untersuchungen zur perkutanen Resorption von Estrogenen und Gestagenen als Grundlage für die Entwicklung eines transdermalen therapeutischen Systems*. Ph. D. Thesis, Freie Universität, Berlin, 1990.
19. B. Bendas, U. Schmalfuß, and R. Neubert. Influence of propylene glycol as cosolvent on mechanisms of drug transport from hydrogels. *Int. J. Pharm.* **116**:19–30 (1995).
20. R. L. Elder. (ed.) Cosmetic Ingredient Review Expert Panel: Final report on the safety assessment of oleic acid, lauric acid, palmitic acid, myristic acid and stearic acid. *J. Am. Coll. Toxicol.* **6**:321–401 (1987).
21. F. A. Andersen. (ed.). Cosmetic Ingredient Review Expert Panel: Final report on the safety assessment of propylene glycol and polypropylene glycols. *J. Am. Coll. Toxicol.* **13**:437–491 (1994).
22. S. Santoyo and P. Ygartua. Effect of skin pretreatment with fatty acids on percutaneous absorption and skin retention of piroxicam after ist topical application. *Eur. J. Pharm. Biopharm.* **50**:245–250 (2000).
23. Y. Komata, A. Kaneko, and T. Fujie. Accumulation of lauric acid in skin as an enhancer for the percutaneous absorption of thiamine disulfide. *Biol. Pharm. Bull.* **18**:791–793 (1995).
24. H.-J. Oh, Y.-K. Oh, and C.-K. Kim. Effects of vehicles and enhancers on transdermal delivery of melatonin. *Int. J. Pharm.* **212**: 63–71 (2001).
25. P. W. Stott, A. C. Williams, and B. W. Barry. Mechanistic study into the enhanced transdermal permeation of a model β-blocker, propranolol, by fatty acids: a melting point depression effect. *Int. J. Pharm.* **219**:161–176 (2001).
26. A. P. Funke. *Transdermale Absorption von hoch lipophilen basischen Antiestrogenen aus Flüssigformulierungen und Matrix-Transdermalsystemen*, Ph. D. Thesis, Freie Universität Berlin, 2000.
27. B. W. Barry. Lipid-protein-partitioning theory of skin penetration enhancement. *J. Control. Release* **15**:237–248 (1991).
28. T. M. Suhonen, J. A. Bouwstra, and A. Urtti. Chemical enhancement of percutaneous absorption in relation to stratum corneum structural alterations. *J. Control. Release* **59**:149–161 (1999).
29. C. S. Leopold and B. C. Lippold. An attempt to clarify the mechanism of the penetration enhancing effects of lipophilic vehicles with differential scanning calorimetry (DSC). *J. Pharm. Pharmacol.* **47**:276–281 (1995).
30. Y. Takeuchi, H. Yasukawa, Y. Yamaoka, K. Taguchi, S. Fukushima, Y. Shimonaka, H. Nishinaga, and Y. Morimoto. Behavior of propylene glycol (PG) in dermis after treatment of rat intact skin surface with fatty acids, fatty amines or azone dissolved in PG. *Biol. Pharm. Bull.* **18**:304–309 (1995).