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Transdermal delivery of levonorgestrel. VIII. Effect of enhancers on rat skin, hairless mouse skin, hairless guinea pig skin, and human skin

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Summary

The penetration enhancing effect of ethyl acetate, with or without ethanol as a cosolvent, was evaluated in vitro on rat skin, hairless mouse skin, hairless guinea pig skin, and human cadaver skin using the contraceptive drug levonorgestrel. Under the influence of the enhancers, the relative skin permeability of levonorgestrel through the four skin types showed a clear trend: hairless mouse skin > hairless guinea pig skin ≥ rat skin > human skin. These results show that rodent skins are poor models for human skin under the conditions used. The steady-state flux of levonorgestrel through human skin was increased about 7-fold (0.03 $\mu g/cm^2 h$ vs 0.2 $\mu g/cm^2 h$) when neat ethyl acetate was used in place of ethanol as the donor vehicle. Adding polyethylene glycol 400 (PEG 400; 40% v/v in saline) to the receptor solution increased the steady-state flux of levonorgestrel through human skin only slightly relative to saline as a receptor solution. The lag time of levonorgestrel through human skin was longer relative to that for the rodent skins tested. The flux of ethyl acetate and ethanol through all the skins was also measured. The rodent skins were all much more permeable towards both solvents than was human skin. For each skin type tested, there appeared to be a direct correlation between the cumulative amount of solvent permeating through the skin and the cumulative amount of drug permeating through the skin.

Introduction

Many chemicals are capable of increasing the percutaneous absorption of coadministered drugs (Hadgraft 1984, Barry 1987a, b, Cooper and Berner, 1987, Walters 1988). Recently, it was found that ethyl acetate (EtAc) was an effective penetration enhancer for a number of drugs, including the contraceptive agent levonorgestrel (LN) (Catz and Friend, 1989, Friend et al., 1989). Permeability of LN through rat skin is increased about 10-fold using EtAc as a vehicle relative to using EtOH as a reference vehicle and about 75-fold relative to water (Friend et al., 1989).

It is well known that rodent skins are generally more permeable than is human skin (Wester and Noonan, 1980; Bronaugh et al., 1982; Reifenrath et al., 1984; Wester and Maibach, 1985; Scott et al., 1986). Initial experiments evaluating the percutaneous absorption of LN used the skin of the common laboratory rat (Wistar strain) (Friend et al., 1988a, b, 1989). A preliminary experiment with human skin indicated that with ethanol

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(EtOH) as a penetration enhancer, the flux of LN was about 4 times less through human skin relative to rat skin (Friend, 1988a). The present study was carried out to evaluate the relative skin permeability of LN through different skin types using the penetration enhancers EtAc and EtOH. A comparison of the skin permeability of LN and the enhancers through excised rat, hairless mouse, hairless guinea pig, and human skin is presented herein.

Materials and Methods

Materials

LN (micronized) was a gift from the World Health Organization. EtAc (U.S.P./N.F.) and absolute EtOH (U.S.P./N.F.) were obtained from Spectrum Manufacturing Corp. (Gardena, CA). Polyethylene glycol 400 (PEG 400) was purchased from Aldrich Chemical Co. (Milwaukee, WI). All other solvents were reagent grade and were used as received.

The rats (male Wistar strain; 8 to 10 weeks old; 180 to 220 g) were obtained from Simonsen Labs, Gilroy, CA. The hairless mice (male HRS/J strain; 8 to 10 weeks old; 20 to 25 g) were obtained from Jackson Labs, Bar Harbor, ME. The hairless guinea pigs (male IAF/HA-HO strain; 8 weeks old, 300 to 350 g) were obtained from Charles Rivers Laboratories, Wilmington, MA. Human cadaver skin was obtained at autopsy through the Stanford University Medical Center. The skin was excised using a dermatome from the thigh area of two females within 24 h post-mortem at a thickness of 100 to 150 μm as measured by a Van Keuren light wave micrometer (L.S. Starrett Co., Athol, MA). The cadaver skin was rinsed with pH 7.4 phosphate buffered saline and then blotted dry. The skin was then placed in a plastic bag and the air evacuated. The plastic bag was then sealed and placed inside two other plastic bags, each evacuated of air and then sealed. The skin sealed in the plastic bags was then stored at $-20\,^{\circ}$C; it was used in the permeability experiments within 2 months. Human skin is normally stable when properly stored up to one year (Harrison et al., 1984).

Permeability experiments

A system employing nine glass Franz diffusion cells was used for the permeability experiments. The Franz cells were modified with inlet and outlet receiver phase ports to allow continuous flow through the cell. The rodents were sacrificed in a CO_2 chamber, and an approximately 3 to 6 cm^2 area of full-thickness skin was excised from the abdomen. The abdominal region of the rats was shaved with electric clippers prior to killing. After removal of the subcutaneous fat, the skins were washed with physiological saline and used in the permeability experiment within 1 h. The skin was mounted and clamped between the cell body and the cell cap with the epidermal side facing upward (donor side). The surface area exposed to the donor phase was 5.07 cm^2 (rat skin), or 2.0 cm^2 (hairless mouse, hairless guinea pig, human cadaver skin). The donor phase (approx. 3 to 5 ml) was prepared by suspending excess solid LN in the appropriate solvent. The donor phase suspension was applied directly on the skin through the cell cap, which was then sealed with a glass stopper. The receptor phase, in contact with the underside of the skin, was isotonic saline at $37\,^{\circ}$C with 0.05% sodium azide added to prevent bacterial growth. In one set of cells used with the human skin, 40% PEG (v/v) was added as a solubilizing agent in the receptor phase. The cells were maintained at $37\,^{\circ}$C by thermostatically controlled water which was circulated through a jacket surrounding the cell body. The donor phase temperature was measured at $32\,^{\circ}$C.

Receiver phase solution was pumped through the diffusion cell by means of a Manostat Cassette Pump drive unit. A fraction collector was used to collect the cell effluent. The flow rate was set so that the drug concentration in the receptor phase remained below about 10% of saturation; a typical flow rate was 10 ml/h. Uniform mixing of the drug in the receiver phase was achieved by a small magnetic stirring bar driven by an external 600 rpm motor. The donor suspensions were changed daily to reduce the possibility of dilution with water, which can enter the donor phase by back-diffusion from the receptor phase. Fractions were collected every 2 h in test tubes. Flux was calculated by measuring the total amount of LN and

solvent collected in the 2 h period, then dividing by 2 to obtain an hourly rate.

The donor phases tested were neat EtAc, neat EtOH, and various mixtures (0.3, 0.5, and 0.7 volume fractions of EtAc in EtOH). All vehicles were tested in triplicate for each skin type.

Chromatographic analysis

LN concentration in the receptor phase was measured using HPLC. No sample pretreatment was required. The HPLC analyses were performed on a Waters 840 system consisting of two Model 510 pumps, a Model 481 UV detector, a Model 710B WISP (sample processor), and a Digital Computer Model 350 microprocessor/programmer. The column used to separate LN was a 4.6 mm × 25 cm, 10 μm, Whatman ODS-3 Partisil C-18. LN was measured with a mobile phase of acetonitrile/H_2O (50 : 50; v/v) at a flow rate of 2.0 ml/min with absorbance monitoring at 243 nm. The retention time of LN was 6.0 min.

EtOH and EtAc were measured in the receptor phase with a Waters Fast Fruit Juice Column (7.8 mm × 15 cm). The mobile phase used was 0.05% H_3PO_4 in H_2O (v/v) at a flow rate of 1.5 ml/min. EtAc and EtOH were detected with a Waters R-400 Differential Refractometer. The retention time of EtOH was 4.2 min while that of EtAc was 6.6 min. Standards of EtAc and EtOH were used to correct for the loss of these two volatile solvents from the effluent collected in the test tubes on the fraction collector prior to analysis by HPLC.

Results

The transdermal flux of LN from saturated solutions of EtOH, EtAc, and EtAc/EtOH mixtures was determined using four types of skin: rat, hairless mouse, hairless guinea pig, and human. The flux of the solvents through the four skin types was also determined.

The flux of LN through excised rat skin using EtAc/EtOH (0.3 : 0.7 and 0.7 : 0.3 volume fractions) is shown in Fig. 1. The steady-state flux from the EtAc/EtOH (0.7 : 0.3) was 2 to 3 μg/cm^2 h, which was reached in about 20 h. LN flux from the EtAc/EtOH (0.3 : 0.7) donor solvent system

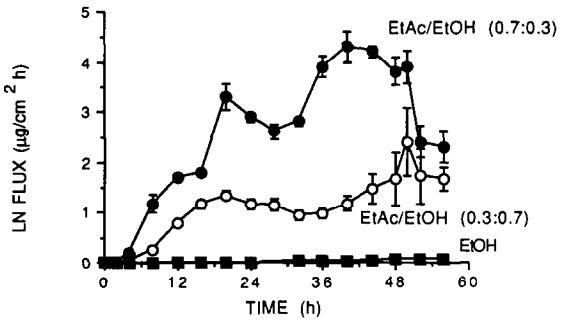

Fig. 1. Flux of LN through rat skin using EtAc/EtOH (0.7 : 0.3; $n = 3$), EtAc/EtOH (0.3 : 0.7; $n = 3$), and neat EtOH ($n = 3$) as donor vehicles. Error bars are mean standard error.

was about 1.2 μg/cm^2 h. The flux of LN in some of the experiments exhibited considerable scatter at various times. Such scatter, not nearly as evident in cumulative plots, is typical in experiments of this type. The flux of EtAc from the two donor solvent systems through rat skin is shown in Fig. 2. The steady-state flux was about 10 mg/cm^2 h and 3 mg/cm^2 h from the EtAc/EtOH (0.7 : 0.3) and EtAc/EtOH (0.3 : 0.7) donor solvents, respectively. EtOH delivery through rat skin from these donor solvents is shown in Fig. 3. The delivery of EtOH from EtAc/EtOH (0.3 : 0.7) was still less than that from the EtAc/EtOH (0.7 : 0.3) despite the fact that there was more EtOH in the EtAc/EtOH (0.3 : 0.7) donor solvent. The flux data and lag times for delivery of LN, EtAc, and EtOH are shown in Table 1 for all four skin types tested.

The transdermal flux of LN through excised hairless mouse (HM) skin from EtAc/EtOH

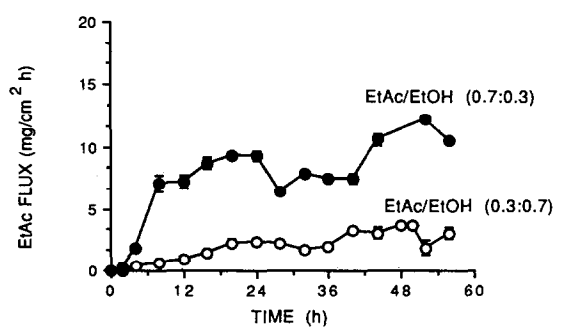

Fig. 2. Flux of EtAc through rat skin using EtAc/EtOH (0.7 : 0.3; $n = 3$), and EtAc/EtOH (0.3 : 0.7; $n = 3$) as donor vehicles. Error bars are mean standard error.

TABLE 1

Lag times and steady-state fluxes of levonorgestrel, ethyl acetate, and ethanol through rat skin, hairless mouse skin, hairless guinea pig skin, and human skin

Skin type	Vehicle [a]	Lag time (h)			Steady-state flux [b]		
		LN	EtAc	EtOH	LN	EtAc	EtOH
Rat	EtOH	16	NA [c]	20	0.06	NA	ND [e]
	EtAc/EtOH (0.3 : 0.7)	16	16	22	1.2	3	7
	EtAc/EtOH (0.5 : 0.5)	20	12	14	3.5	5	9
	EtAc/EtOH (0.7 : 0.3)	20	8	12	3.0	10	9
	EtAc	14	8	NA	1.0	12	NA
HM	EtAc/EtOH (0.3 : 0.7)	6 [d]	6	8	4.4 [d]	8	20
	EtAc/EtOH (0.5 : 0.5)	4 [d]	6	4	10 [d]	13	16
	EtAc/EtOH (0.7 : 0.3)	6 [d]	6	8	4.1 [d]	18	17
HGP	EtAc/EtOH (0.3 : 0.7)	20	20	22 [d]	1.1	1	4 [d]
	EtAc/EtOH (0.5 : 0.5)	12	10	12	1.3	3	4
	EtAc/EtOH (0.7 : 0.3)	12	8	16	2.3	7	11
Human	EtOH	32	NA	12	0.03	NA	1.5
	EtAc/EtOH (0.3 : 0.7)	22	18	12	0.08	0.3	1.1
	EtAc/EtOH (0.5 : 0.5)	22	20	12	0.12	0.5	1.0
	EtAc/EtOH (0.7 : 0.3)	22	20	12	0.12	0.5	1.1
	EtAc	26	24	NA	0.20	0.5	NA
	EtAc (PEG 400)	26	20	NA	0.25	0.5	NA

[a] Expressed as volume fractions; [b] expressed as $\mu g/cm^2$ h for levonorgestrel and mg/cm^2 h for ethyl acetate and ethanol; [c] NA = not applicable; [d] steady-state conditions were not maintained; [e] ND = not determined.

(0.7 : 0.3 and 0.3 : 0.7 volume fractions) is shown in Fig. 4. The flux of LN did not reach a steady-state condition from either of these vehicles. A peak flux of LN of about 10 $\mu g/cm^2$ h was measured using a donor vehicle of EtAc/EtOH (0.5 : 0.5 volume fractions). This was the highest flux of LN observed through any of the skins tested. The delivery of solvent through HM skin was also greater and more immediate than observed for the other skins as is shown in Figs. 5 and 6 for EtAc and EtOH, respectively. Solvent delivery through HM skin was relatively constant once steady-state was reached.

The third rodent skin examined in this study was obtained from the hairless guinea pig (HGP), which has been recently suggested as a good model

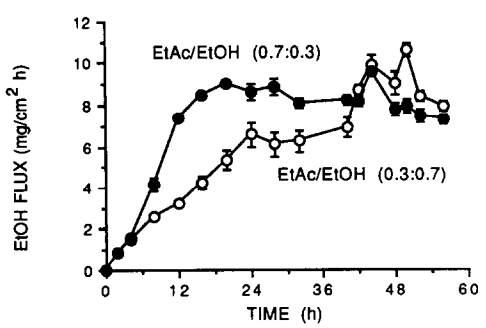

Fig. 3. Flux of EtOH through rat skin using EtAc/EtOH (0.7 : 0.3; $n = 3$), EtAc/EtOH (0.3 : 0.7; $n = 3$) as donor vehicles. Error bars are mean standard error.

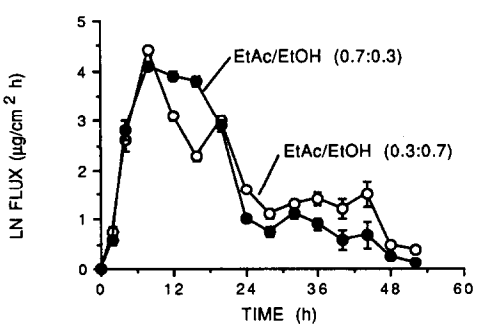

Fig. 4. Flux of LN through HM skin using EtAc/EtOH (0.7 : 0.3; $n = 3$), EtAc/EtOH (0.3 : 0.7; $n = 3$) as donor vehicles. Error bars are mean standard error.

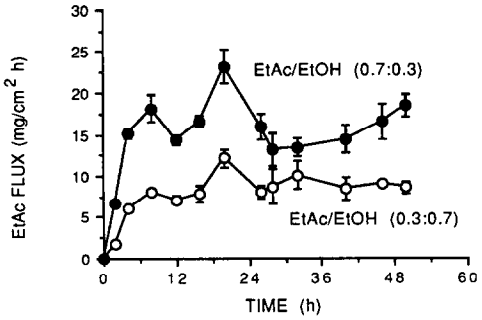

Fig. 5. Flux of EtAc through HM skin using EtAc/EtOH (0.7 : 0.3; $n = 3$), EtAc/EtOH (0.3 : 0.7; $n = 3$) as donor vehicles. Error bars are mean standard error.

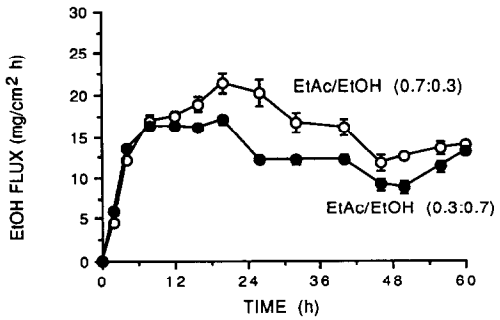

Fig. 6. Flux of EtOH through HM skin using EtAc/EtOH (0.7 : 0.3; $n = 3$), EtAc/EtOH (0.3 : 0.7; $n = 3$) as donor vehicles. Error bars are mean standard error.

for human skin (C. Behl, personal communication). The flux of LN from EtAc/EtOH solvent systems (0.7 : 0.3 and 0.3 : 0.7) is shown in Fig. 7.. The steady-state flux of LN was reached in about 12 to 20 h, depending on the donor phase. The

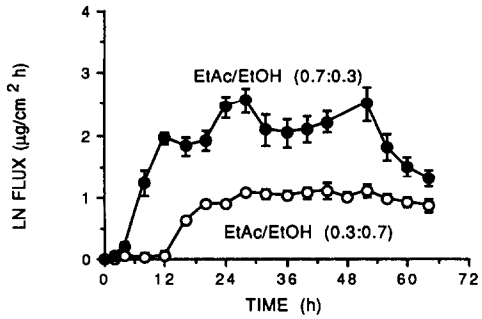

Fig. 7. Flux of LN through HGP skin using EtAc/EtOH (0.7 : 0.3; $n = 3$), EtAc/EtOH (0.3 : 0.7; $n = 3$) as donor vehicles. Error bars are mean standard error.

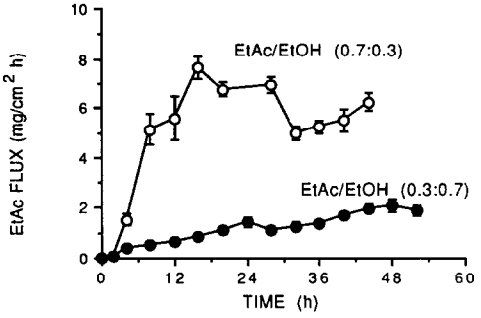

Fig. 8. Flux of EtAc through HGP skin using EtAc/EtOH (0.7 : 0.3; $n = 3$), EtAc/EtOH (0.3 : 0.7; $n = 3$) as donor vehicles. Error bars are mean standard error.

delivery of EtAc and EtOH from these two donor solvent systems is shown in Figs. 8 and 9, respectively. The steady-state flux of EtAc was about 7 mg/cm^2 h (0.7 EtAc/0.3 EtOH) and about 1 mg/cm^2 h (0.3 EtAc/0.7 EtOH), both of which were close to the measured flux of EtAc and EtOH through rat skin from the same vehicles.

The flux of LN through excised human cadaver skin using EtAc/EtOH solvent systems (0.7 : 0.3 and 0.3 : 0.7) is shown in Fig. 10. The lag times for delivery of LN through human skin were longer than observed with the rodent skins. These lag times (22–26 h) are similar to those observed for delivery of LN through human skin in vitro from transdermal devices which use other chemical enhancers (Chien et al., 1988). The flux of LN through human skin from the 0.3 EtAc/0.7 EtOH donor vehicle at steady-state was about 0.08 µg/cm^2 h and about 0.12 µg/cm^2 h from the 0.7

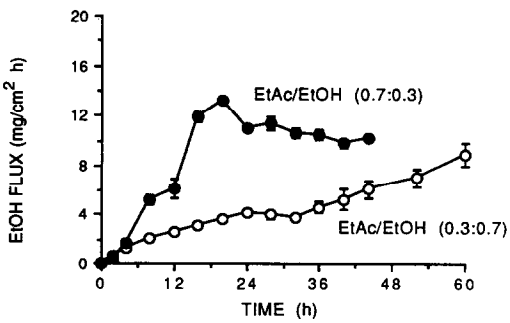

Fig. 9. Flux of EtOH through HGP skin using EtAc/EtOH (0.7 : 0.3; $n = 3$), EtAc/EtOH (0.3 : 0.7; $n = 3$) as donor vehicles. Error bars are mean standard error.

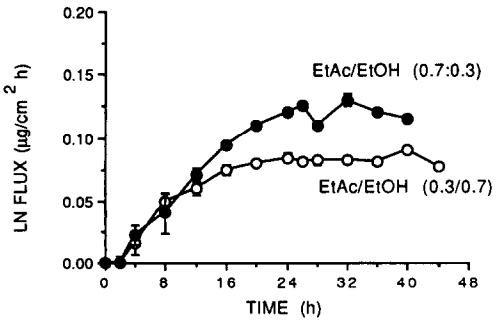

Fig. 10. Flux of LN through human skin using EtAc/EtOH (0.7:0.3; $n = 3$), EtAc/EtOH (0.3:0.7; $n = 3$) as donor vehicles. Error bars are mean standard error.

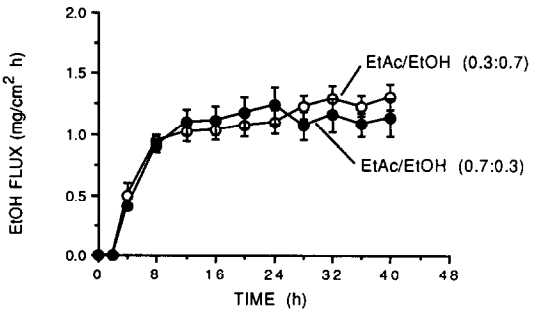

Fig. 12. Flux of EtOH through human skin using EtAc/EtOH (0.7:0.3; $n = 3$), EtAc/EtOH (0.3:0.7; $n = 3$) as donor vehicles. Error bars are mean standard error.

EtAc/0.3 EtOH donor vehicle. These fluxes were considerably less than was observed through the rodent skins using the same donor solvents (see Table 1). The flux of LN through human skin using neat EtAc as a donor vehicle was higher than was measured using the EtAc/EtOH cosolvent systems: 0.2 $\mu g/cm^2$ h. This value is about 5 times less than that through rat skin from a neat EtAc donor vehicle (see Table 1). The flux of LN through human skin using neat EtOH as a donor vehicle was about 0.03 $\mu g/cm^2$ h, close to that reported previously (Friend et al., 1988a).

The permeability of human skin to EtAc (Fig. 11) was considerably less than was observed through the rodent skins for all the EtAc-containing vehicles. The flux of EtAc ranged between 0.3 and 0.5 mg/cm^2 h, depending on the vehicle composition. The flux of EtOH through human skin from these two donor vehicles is shown in Fig. 12. The steady-state flux (1.0 to 1.5 mg/cm^2 h) was nearly identical for all the EtOH-containing vehicles; lag times (about 12 h) were about the same as well.

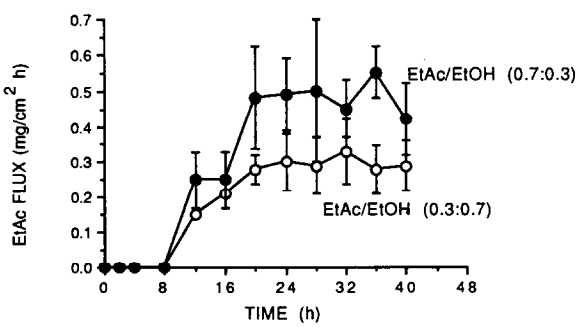

Fig. 11. Flux of EtAc through human skin using EtAc/EtOH (0.7:0.3; $n = 3$), EtAc/EtOH (0.3:0.7; $n = 3$) as donor vehicles. Error bars are mean standard error.

PEG 400 has been used as a solubilizing agent in the receptor solution of in vitro permeability experiments (Valia et al., 1984). PEG 400 (40% v/v in saline) was used as a solubilizing agent in the receptor phase using neat EtAc as an enhancer with human skin. The flux was slightly higher using PEG 400 relative to normal saline as a receptor solution. The flux of EtAc was unaffected by the PEG. Information from this experiment is summarized in Table 1.

The relationship between drug flux and solvent flux was examined by comparing cumulative drug and solvent delivery. This was done for the EtAc/EtOH (0.7:0.3) donor vehicle with all four skin types (see Fig. 13). It appears that there is a nearly linear relationship between cumulative drug delivery and cumulative solvent delivery regardless of the skin type. There is a deviation from this relationship with the HM skin, which is due to the drop in drug delivery after 12 h as shown in Fig. 4. The drop in LN flux through HM skin may have been caused by water entering the donor phase from the receptor solution through back-diffusion leading to an alteration of the penetration enhancing effect of the solvents. Back-diffusion of water into the donor chamber has been observed by others under conditions similar to those used herein (Allenby et al., 1969; Coldman et al., 1971; Ito et al., 1988). However, the donor phase was replaced at 24 h with no change in the flux as can

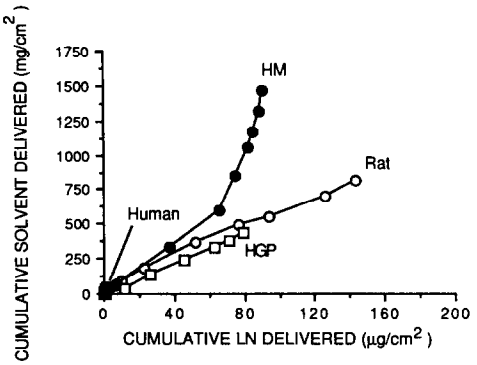

Fig. 13. Relationship between cumulative LN delivered and cumulative total solvent delivered through rat skin, HM skin, HGP skin, and human skin using a donor vehicle of EtAc/EtOH (0.7:0.3).

be seen in Fig. 4. This indicates that the change in flux over time may have been due to changes in the skin. There was no change over time in the steady-state permeation of EtAc and EtOH through the HM skin. For comparison purposes using HM skin, the flux over the first 12 to 18 h was used. After that time, the flux of LN fell considerably and was therefore not used as this phenomenon was unique to HM skin.

Discussion

The development of a small (5- to 10-cm^2), once-a-day transdermal delivery system for LN requires a very effective penetration enhancer. EtAc, either alone or in combination with EtOH, meets this requirement. Other enhancers found effective at increasing the skin permeability of LN and hydrophobic compounds in general are L-α-amino acids (Sarpotdar et al., 1988) and mixtures of a base vehicle such as propylene glycol and small quantities of fatty acids (Cooper, 1984). Preliminary enhancer studies with EtAc involved the use of rat skin, which is more permeable than is human skin (Bronaugh et al., 1982; Reifenrath et al., 1984; Scott et al., 1986; Wester and Noonan, 1980; Wester and Maibach, 1985). The present studies were performed to assess the ability of EtAc to enhance the skin permeability of LN through human skin as well as through several other rodent skins.

The relative permeability of the skins tested indicates that HM skin is significantly more permeable towards LN compared to the other rodent skins using combinations of EtAc/EtOH as enhancers. All of the rodent skins were more permeable than was human skin toward LN, EtAc, and EtOH. The relative permeability of LN through human skin using neat EtAc as an enhancer was about 5 times less than that of rat or HGP skin. Clearly, these results indicate that the rodent skins are not useful for direct comparisons with human skin in permeability experiments. In particular, HM skin appears to considerably overestimate the penetration enhancing effect of EtAc for LN. HM skin has been criticized as a model for human skin particularly in evaluation of the effect of chemical enhancers (Bond and Barry, 1988a, b). A problem with HM is its susceptibility to hydration effects (Behl et al., 1980). Rat skin and HGP are probably better models for human skin than is HM skin. For example, rat skin does not appear to suffer from hydration effects as does HM skin (Catz and Friend, 1989). Nonetheless, all three rodent skins overestimate absolute drug flux under the influence of the penetration enhancers EtAc and EtOH and should therefore be used with caution in making predictions about human skin.

Full-thickness rodent skins (epidermis and dermis) were used in these experiments. It is known that full-thickness skin, when tested in vitro with hydrophobic compounds, may underestimate the permeability of the same skin in vivo (Tsuruta, 1977; Bronaugh and Stewart, 1984, 1986). This is because compounds of very low water solubility (e.g., LN) do not partition freely from the stratum corneum into the aqueous environment of the viable epidermis or the aqueous receptor fluid. In vivo, the drug is absorbed by the blood in the capillaries that lie at a depth of about 200 μm from the surface of the skin (Schaefer et al., 1982, Barry, 1983).

A technique used to obviate the problem of poor in vitro/in vivo correlation when using full thickness skin in vitro to evaluate hydrophobic compounds is to add solubilizing agents to the receptor solution. Examples of solubilizing agents used to increase drug solubility in the receptor

solution include PEG-20 oleyl ether, octoxynol-9 (Triton X-100) (Bronaugh and Stewart, 1984), bovine serum albumin (3% in buffer) (Brown and Ulsamer, 1975), Poloxamer 188 (Hoelgaard and Mollgaard, 1982), and PEG 400 (Valia et al., 1984). We tested PEG 400 (40% in saline) as a solubilizing agent with human skin using neat EtAc as an enhancer. The results indicated that the percutaneous absorption of LN was increased only slightly by this change. This may have been due to the fact that the human skin used was relatively thin (100–150 μm thick) which would reduce the thickness of the unstirred water layer. Another possibility is that EtAc and EtOH act as solubilizing agents themselves as they enter the skin and pass through the viable epidermis, then the dermis, and finally into the receptor solution. Therefore, the addition of PEG 400 to the receptor solution when EtAc and EtOH are used as enhancers might have little or no effect. The flux of EtAc through the human skin using PEG 400 in the receptor medium was unchanged relative to that using saline as a receptor medium indicating that the PEG 400 did not measurably alter the barrier properties of the skin. However, the concentrations of EtAc in the receptor solutions were very close to the detection limits of the HPLC technique making small changes in concentration difficult to measure. Sarpotdar and coworkers observed that PEG 400 can have a significant effect on the penetration barrier of human cadaver skin in vitro (Sarpotdar et al., 1986).

EtAc is hydrolyzed to EtOH and acetic acid in plasma by nonspecific esterases (Gallaher and Loomis, 1975). The viable epidermis also contains esterases capable of hydrolyzing ester-containing compounds (Pannatier et al., 1981; Bucks, 1984). When neat EtAc was used as a donor vehicle with rat, HM, and HGP skins, there were measurable amounts of EtOH detected in the receptor solutions. EtOH in the receptor solutions ranged from 5–20% by weight of the total EtAc measured in the receptor solutions at steady-state. This represents approximately half the amount of hydrolyzed EtAc as only ethanol was measured in these experiments. Thus, there appears to be considerable hydrolysis of EtAc as it passes through the viable epidermis and dermis. EtAc in the donor phase is stable over the course of the experiment. Hydrolysis of EtAc may also be due to esterases released into the receptor medium over the course of the experiment as has been reported for human skin (Bundgaard et al., 1983; Hoelgaard and Mollgaard, 1985). There was no EtOH detected in the receptor solutions when human skin was tested with neat EtAc as a donor vehicle. Esterase activity was probably abolished by the freeze/thaw cycle that the human skin was subjected to prior to performance of the permeability experiment. Also, since small amounts of EtAc were found to permeate through human skin, any EtOH generated from hydrolysis was probably below the detection limit for EtOH (less than 200 μg/ml). The flux values of EtAc and EtOH as reported in Table 1 were calculated from the measured concentration of EtAc and EtOH in the receptor solutions and are uncorrected for metabolism.

Local concentrations of EtAc and EtOH are probably high enough in the stratum corneum and the viable tissues to increase the solubility of LN in the skin (and receptor solutions). This would alter partitioning of LN between the vehicle and the stratum corneum (and perhaps the viable tissues) causing an increase in drug flux. As shown in Fig. 13, the amount of drug permeating throughthe skin appears to be related directly to the amount of solvent permeating through the skin lending support to the idea that these solvents are increasing partitioning of LN into the stratum corneum and possibly into the viable tissues. At the same time, differential scanning calorimetry and Fourier transform infrared spectroscopic studies indicate the EtAc extracts measurable amounts of lipid from both hairless mouse and human stratum corneum (unpublished results). Such physical alteration of the barrier (as well as potential alterations in lipid fluidity or disruption of the keratin fibrils of corneocytes) would increase diffusion of drugs through the stratum corneum and hence increase overall drug flux. Most of the experiments with LN (Friend et al., 1989) indicate that the lag times of LN through the various skins are not significantly reduced by the enhancers EtAc and EtOH. These results would indicate that penetration enhancement is due primarily to changes in partitioning (Guy and

Hadgraft, 1988). Clearly, further experiments are required to determine the specific skin penetration enhancing effects of EtAc and EtOH.

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