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ENHANCEMENT OF *IN VITRO* SKIN PERMEATION OF VERAPAMIL

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ABSTRACT

A number of compounds including aliphatic esters, alkanols and amides were investigated for their ability to enhance the *in vitro* permeation rate of verapamil across hairless mouse skin. While several of them did enhance the permeation rate, the best results were obtained with the lauric acid derivatives. On analysis of the permeation rate data it was found that while most of the compounds increased the solubility of verapamil in skin and hence its partition coefficient from the delivery system, the lauric acid derivatives also increased its diffusivity in skin. Permeation of verapamil from delivery systems containing these lauric derivatives was further investigated through delipidized and stripped skin. It was found that most of these derivatives enhanced the permeation of verapamil across both whole and delipidized skin, indicating that in addition to their action on the skin lipids, they must also have some effect on the proteins in the stratum corneum.

INTRODUCTION

Due to the increasing interest in and the growing popularity of transdermal drug delivery systems, there is a constant search for substances which would improve the delivery of topically applied drugs into the systemic circulation. A "penetration/ permeation enhancer" can be defined as an agent which reduces the diffusional resistance of the stratum corneum by reversibly damaging it or by altering its physicochemical nature[1]. Several classes of compounds have been studied as potential permeation enhancers, including surfactants, like sodium lauryl sulfate[2]; sulfoxides, like dimethyl sulfoxide[3]; glycols, like propylene glycol[4]; pyrrolidones, like 2-pyrrolidone[5]; fatty acids, like oleic acid[6]; fatty alcohols, like lauryl alcohol[7]; and amides and amines, like dimethyl-formamide[5,7]; and recently, a novel compound, Azone[8].

Verapamil is a calcium channel blocker, used in the treatment of arrhythmias, angina and hypertension[9]. Due to its poor bioavailability after oral administration and short plasma half life, it would make a good candidate for transdermal delivery. We[10] have previously reported the development and clinical evaluation of a transdermal drug delivery system for verapamil using silicone elastomers as the polymer matrix and isopropyl myristate (IPM) as permeation enhancer. The *in vivo* data indicated that a higher skin permeation rate would be necessary in order to achieve effective therapeutic plasma levels (50-100 ng/ml) of verapamil.

The objectives of this investigation were to investigate a number of compounds for their ability to enhance the *in vitro* skin permeation rate of verapamil. An attempt was also made to evaluate the possible mechanisms of action of these compounds.

MATERIALS AND METHODS

Materials

Sulfuric acid, acetonitrile and PEG 400 were purchased from Fisher Scientific. Myristic acid methyl ester, myristic acid ethyl ester, myristic acid propyl ester, myristic acid arachidyl ester, myristic acid behenyl ester, caproic acid propyl ester, caprylic acid propyl ester, capric acid propyl ester, lauric acid propyl ester,

palmitic acid propyl ester, stearic acid propyl ester, lauric acid methyl ester, lauric acid ethyl ester, lauric acid butyl ester, n-octyl acetate, n-decyl acetate, n-lauryl acetate, myristyl acetate, butyl alcohol, hexyl alcohol, octyl alcohol, decyl alcohol, lauryl alcohol, cetyl alcohol, caproic acid N,N-dimethylamide, lauric acid N,N-dimethylamide, myristic acid N,N-dimethylamide, 2-pyrrolidone, N-methyl-pyrrolidone and N-ethylpyrrolidone were used as obtained from Sigma Chemicals. Silicone elastomers, DC-382 and DC-360 were donated by Dow Corning Corp.

Transdermal Drug Delivery Systems

Transdermal drug delivery systems (TDDS) were fabricated by dispersing appropriate amounts of verapamil and permeation enhancer in silicone elastomer, DC-382, containing 10% w/w of silicone fluid, DC-360 (100 cs viscosity). The components were well mixed using a lab stirrer (Cole Palmer Model 4380-00) at a speed setting of 7. The requisite amount of Catalyst M (stannous octanoate) was added, mixed well and then degassed under vacuum. The mixture was then spread between sheets of backing laminate, placed in a stainless steel compression mold and cured in an oven at 60°C. Unless otherwise mentioned, the verapamil loading dose was kept constant at a level of 25% (w/w), while the permeation enhancers were incorporated at a concentration of 10% (w/w).

Skin Permeation Procedure

For most of the experiments, freshly-excised abdominal skin specimens from 6-8 weeks female hairless mice, HRS/J strain (Jackson Laboratories, Maine) were used. The skin specimen was mounted on each half-cell of a side-by-side permeation system[11] with the dermis side facing the receptor compartment. The temperature in the receptor compartment was maintained at 37°C. A unit of verapamil-TDDS (1 cm^2) was then placed on the stratum corneum surface of each skin specimen and the two half-cells were then held together tightly by a clamp. Each half-cell contained 3.5 ml of a 40% PEG 400 aqueous solution as receptor fluid. At predetermined time intervals, 50 μl of the receptor solution was sampled, diluted with 1 ml of HPLC grade water and assayed for verapamil concentration by HPLC.

For experiments involving stripped skin, the skin while still on the carcass of the animal was stripped repeatedly (15 times) of its stratum corneum using

adhesive tape (SCOTCH tape). For experiments involving delipidized skin, the freshly-excised skin of hairless mouse was mounted with the stratum corneum facing the donor compartment and clamped tightly between the two half-cells of each skin permeation system. Methylene chloride (3.5 ml) was placed in the donor compartment, while the receptor compartment was empty. Both half-cells were stoppered to prevent solvent loss by evaporation. After extraction, with stirring, for four hours, the methylene chloride was withdrawn and discarded. Trace amount of methylene chloride which remains in the donor compartment or on the skin surface was evaporated under a stream of air. The skin permeation experiment was then carried out as described above.

All experiments were carried out in triplicate.

Solubility of Verapamil in the TDDS

Solubility of verapamil in silicone fluid with or without an enhancer was determined by equilibrating an excess of the drug with the appropriate vehicle in a shaking water bath (Fisher Model 129) at 37°C for 24 hr. In case of an enhancer-containing silicone fluid, the appropriate concentration of enhancer was dissolved in silicone fluid prior to the solubility determinations. At the end of 24 hr, 1 ml aliquot was withdrawn and extracted with 10 ml of methanol, using a wrist action shaker (Burrell Model 75), for 12 hr. The methanol extracts were then appropriately diluted and analyzed by HPLC.

All solubility determinations were carried out in triplicate.

Analytical Procedure

Samples containing verapamil were analyzed by a modification of the HPLC procedure developed by Harapat and Kates[12]. A Waters Tri-Module system with a Kratos Model 783 variable wavelength UV detector was used for the verapamil assay. A Waters μBondapak C_{18} column (15 cm x 3.9 mm I.D.) was used in combination with a mobile phase consisting of water (adjusted to pH 2.4 with sulfuric acid) and acetonitrile, in the proportion 40:60. The flow rate was 2 ml/min and verapamil was detected at 232 nm.

DATA TREATMENT

The skin permeation rate, **J**, was calculated from the slope of the linear plot of the cumulative amount permeated as a function of time in the steady-state

region. The lag time, t_L, was determined from the time-axis intercept of the extrapolation of this linearity. The enhancement factor, **E**, was calculated as the ratio of the permeation rate of verapamil from a delivery system containing enhancer to that from a control delivery system which did not contain any permeation enhancer.

In order to evaluate the possible mechanisms of action of the permeation enhancers, the permeation rate data were further evaluated as follows. The permeability coefficient, **P**, was calculated from the following relationship:

$$P = \frac{J}{C_d} \quad (1)$$

where C_d is the solubility of verapamil in the donor (delivery system). The effective diffusivity, **D**, is a function of the lag time, t_L, and can be calculated as follows:

$$D = \frac{h^2}{6t_L} \quad (2)$$

The permeability coefficient, **P**, is actually described by the following relationship:

$$P = \frac{K.D}{h} \quad (3)$$

where **K** is the effective partition coefficient of verapamil between the homogeneous skin and the delivery system and **h** is the thickness of whole skin. Rearranging Equation 3, we get:

$$K = \frac{P.h}{D} \quad (4)$$

Since all the quantities on the right hand side of Equation 4 can be determined, the partition coefficient, **K**, can be calculated.

RESULTS AND DISCUSSION

The values of **E**, **P**, **D** and **K**, for the various enhancers studied are listed in Table I.

Table 1

Effect of Various Permeation Enhancers on the Enhancement Factor, E, Permeability Coefficient, P, Diffusivity, D, and Partition Coefficient, K, of Verapamil in Hairless Mouse Skin.

Permeation Enhancer	E	P x 10^{-6} (cm/s)	D x 10^{-8} cm^2/s	K
Propyl Esters of Aliphatic Acid				
Caproic acid	1.2	5.6	1.3	15.6
Caprylic acid	1.5	6.1	1.7	13.1
Capric acid	1.7	3.9	1.3	11.3
Lauric acid	1.8	4.4	3.9	4.2
Myristic acid	1.0	2.7	1.3	7.6
Palmitic acid	0.8	2.3	1.6	5.3
Stearic acid	0.9	5.1	2.0	9.3
Myristic Acid Esters				
Methyl ester	2.1	7.0	1.9	13.4
Ethyl ester	2.2	6.3	1.9	12.2
Propyl ester	1.0	2.7	1.3	7.6
Lauric Acid Esters				
Methyl ester	1.8	4.3	2.4	6.6
Ethyl ester	2.3	5.6	2.5	8.3
Propyl ester	1.8	4.4	3.9	4.2
Butyl ester	1.1	4.2	1.7	9.3

Table I contd.

Permeation Enhancer	E	P x 10^{-6} (cm/s)	D x 10^{-8} cm^2/s	K
Aliphatic Acetates				
n-Octyl acetate	1.3	2.9	2.4	4.4
n-Decyl acetate	1.1	2.6	2.0	4.7
n-Lauryl acetate	0.8	1.8	3.8	1.8
Myristyl acetate	1.6	3.6	2.2	6.3
Aliphatic Alcohols				
Butyl alcohol	0.6	1.1	3.3	1.2
Hexyl alcohol	0.5	1.1	5.3	0.8
Octyl alcohol	0.7	1.6	2.6	2.3
Decyl alcohol	0.9	2.6	3.0	3.3
Lauryl alcohol	1.7	6.5	3.0	8.1
Cetyl alcohol	0.2		2.1	
N,N-dimethylamides of Aliphatic Acids				
Caproic acid	1.1	2.6	1.5	6.6
Lauric acid	2.3	7.0	1.5	17.3
Myristic acid	2.3	11.5	2.0	21.7
N-substituted 2-Pyrrolidones				
Hydrogen	0.6	12.7	3.3	14.2
n-Methyl	0.9	22.6	3.9	21.6
n-Ethyl	1.0	39.2	2.9	49.8

Effect of Aliphatic Acid Esters

Effect of Aliphatic Acid Chain Length: Propyl esters of a series of aliphatic acids were investigated for their ability to enhance the skin permeation rate of verapamil. The enhancement in permeation appears to be a function of the chain length of the aliphatic acid with the maximum enhancement achieved at n = 10, that is, lauric acid propyl ester (Table I; Fig. 1). Similar results have been obtained by Chien et al[13] who studied the ability of aliphatic acid propyl esters to enhance the skin permeation rate of indomethacin. In their study, however, the maximum permeation rate was obtained at n = 6-8, with the flux decreasing as the number of methylene groups was further increased.

The effects of alkyl chain length on **P**, **D**, and **K** are shown in Table I and Fig. 2. The diffusivity seemed to remain constant, except for a sharp increase at n = 10. At the same time, except at n = 10, the partition coefficient and permeability coefficient profiles followed each other very closely. Thus, the major effect of these esters is on the partition coefficient of verapamil, that is, they affect the skin lipids and alter its solubility in the skin. This is supported by the results of Sato et al[14] who concluded that aliphatic esters mainly act on the lipids in the stratum corneum. The data in this study indicate that the lauric acid propyl ester may be an exception, because it also affects the diffusivity of verapamil in the skin.

Effect of Ester Group Chain Length: The chain length of the ester group was varied in case of two aliphatic acids, myristic and lauric, and the results are shown in Table I. In case of myristic acid, only the methyl and ethyl esters were able to significantly enhance the skin permeation rate of verapamil, with the ethyl ester increasing the permeation rate to 93 $\mu g/cm^2$-hr. The other esters did not produce a significant change in the skin permeation rate from that of the control delivery system. For the myristic acid esters, all three parameters, **P**, **D** and **K** have a very similar profile, showing a decrease as the chain length of the ester group increases. The results imply that the change in the permeation rate was due to the effects of the myristic acid esters on the partition coefficient of verapamil into the skin and its diffusivity in the skin.

In case of lauric acid too, the highest permeation rate was obtained with the ethyl ester. The permeation rate then decreased as the ester group chain

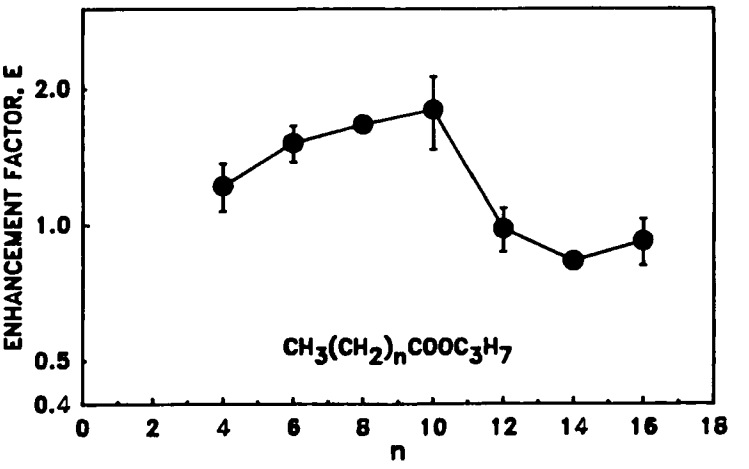

FIGURE 1

Effect of Aliphatic Acid Propyl Esters on the Skin Permeation Rate of Verapamil.

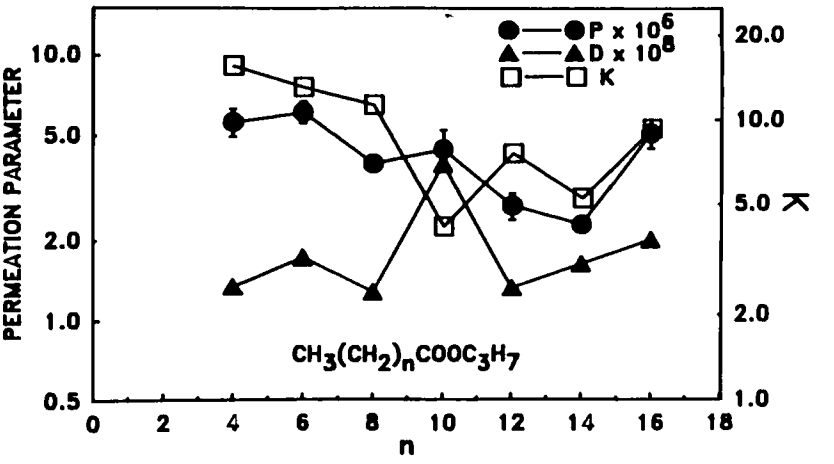

FIGURE 2

Effect of Aliphatic Acid Propyl Esters on the Physicochemical Parameters of Verapamil in Hairless Mouse Skin.

length was further increased. For lauric acid esters **P**, **D** and **K** have very similar profiles for short ester chain length. In the case of lauric acid propyl ester, the diffusivity of verapamil in the skin increased whereas the partition coefficient decreased.

Effect of Aliphatic Acetates

Acetates of four aliphatic acids with chain lengths ranging from 8 through 14 were investigated for their effect on the skin permeation rate of verapamil. They did not enhance the permeation rate substantially, and in the case of lauryl acetate, the rate was even lower than that of the control delivery system (Table I). Interestingly, the maximum permeation rate of 67 $\mu g/cm^2$-hr was obtained with myristyl acetate. In this case too, both **P** and **K** had very similar profiles. Again, the diffusivity increases for the lauryl acetate, while there is a decrease in both the permeation rate and the permeability coefficient.

Effect of Aliphatic Alcohols

The lower alcohols actually suppressed the permeation of verapamil, while the highest permeation rate of 67 $\mu g/cm^2$-hr was again obtained at n = 10, that is the lauryl alcohol (Table I). Aungst et al[7] who studied the effect of fatty alcohols on the permeation of naloxone also obtained the highest flux with lauryl alcohol. Scheuplein and Blank[15] studied the transdermal flux of alkanols themselves from aqueous solutions and found that the permeation rate reached a maxima at n = 6, that is, octanol. The diffusivity of verapamil seems to remain more or less constant in the presence of alkanol. However, both permeability coefficient and partition coefficient increased with the increase in chain length. Durrheim et al[16] found that the permeability coefficient of the alkanols themselves increases linearly with alkyl chain length from C_1 to C_8. Using the data from the literature[15], Chien also showed that a linear relationship exists between the permeability coefficient and the alkyl chain length of alkanols between C_3 and C_8[17].

Effect of Aliphatic Acid N,N-dimethylamides

N,N-dimethylamide derivatives of several aliphatic acids were investigated for their ability to enhance the skin permeation rate of verapamil and the results are shown in Table I. The highest permeation rate of 97 $\mu g/cm^2$-hr was achieved

with lauric acid N,N-dimethyl-amide, although the myristic acid derivative also enhanced the permeation rate to almost the same extent. The diffusivity did not change appreciably, while both permeability coefficient and partition coefficient had similar profiles, increasing with chain length. This means that the change in permeation rates is due to the effect of these dimethylamides on the partition coefficient of verapamil.

Effect of N-substituted-2-Pyrrolidones

N-substituted-2-pyrrolidones did not enhance the skin permeation rate of verapamil. On the contrary, they had an adverse affect, with the permeation rate being below that of the control, except in the case of the ethyl substituent. The permeation rate increased with increasing lipophilicity of the pyrrolidone molecule. The N-substituent has no appreciable effect on the diffusivity of verapamil in the skin, whereas both the permeability coefficient and partition coefficient increase with increasing lipophilicity of the N-substituent. There are several reports about the use of pyrrolidone derivatives as permeation enhancers. For example, Southwell and Barry[5] studied the effects of 2-pyrrolidone on the permeation of water, *n*-alkanols and caffeine. Barry and Bennett[18] found that 2-pyrrolidone and N-methylpyrrolidone enhance the permeation of both mannitol and hydrocortisone. The general conclusion was that the enhancement was greater for polar solutes than for non-polar solutes. This may be the reason why pyrrolidones do not enhance the permeation rate of verapamil, which is relatively more lipophilic. Aungst et al[7] also found that pyrrolidones do not enhance the permeation rate of naloxone.

In general, most of the compounds studied altered the skin permeability of verapamil by affecting its partition coefficient between the skin and delivery system. The stratum corneum, which is the main barrier to skin permeation, is a lipophilic membrane. Solutes can be transported across the skin by at least two pathways: a polar pathway associated with the protein component of the stratum corneum and a nonpolar pathway associated with the lipid component[6]. A change in the partition coefficient represents a change in the solubility of drug in the skin, implying an action on the lipids in the stratum corneum. In other words, these compounds act on the nonpolar pathway in the stratum corneum.

The lauric acid (C_{11}) derivatives were an exception to this behavior, causing an increase in the diffusivity of verapamil. In addition, it is the lauric acid derivatives which caused the highest enhancement in the skin permeation rate of verapamil. Similar results have been obtained with other enhancers like surfactants[7]. Several hypotheses have been proposed to explain why C_{12} hydrophobic groups have maximum effects on the membrane. It has been suggested that increasing the carbon chain length within a homologous series of surfactants increases the lipophilicity, but decreases the critical micelle concentration. C_{12} hydrophobic groups may have the greatest membrane penetration because of an optimal balance of partition coefficient and monomer concentration. Dominguez et al[19] suggested that surfactants do not adopt a linear structure in skin, but rather form a coiled structure. The molecular size of the surfactants forming these coils was calculated to be minimum when the hydrophobic chain was 12 carbons long, which favors membrane penetration. It is also known that lipids of like structure tend to pack tightly together, but mixtures of long and short chain lipids, or saturated and unsaturated lipids, form loosely organized structures[20]. The most abundant lipids in the stratum corneum are free fatty acids, triglycerides, cholesterol, and ceramides. The majority of these lipids have hydrophobic groups with 16 or more carbon atoms[21]. It is possible that the introduction of shorter fatty acid chains disrupts the crystalline lipid packing and results in a more fluid and permeable membrane.

Effect of Delipidization and Stripping

Since the lauric acid derivatives caused the highest enhancement in skin permeation and they also seemed to increase the diffusivity of verapamil, they were selected for further investigation. Lipids are a major constituent of the stratum corneum and form the rate-limiting barrier for many solutes which penetrate via the lipid pathway[22]. Removal of the lipids by extraction is known to reduce the barrier property of the stratum corneum[2]. Permeation through the delipidized skin would provide some insights into the role of lipids in percutaneous absorption. Stripping the skin with adhesive tape can completely remove the stratum corneum to demonstrate to what extent it is the barrier to permeation.

Table II

Effect of Lauric Acid Derivatives on the Permeation Rate of Verapamil through Various Skin Tissues of Hairless Mouse.

Enhancer	Permeation Rate ($\mu g/cm^2$-hr) ± S.D.		
	Whole Skin	Delipidized Skin	Stripped Skin
Control	42.3 ± 7.7	104.5 ± 27.8	263.9 ± 46.5
Lauryl Alcohol	69.6 ± 18.6	155.5 ± 38.2	297.2 ± 69.8
Lauric Acid Derivatives			
Propyl Ester	76.2 ± 13.9	197.6 ± 26.9	323.1 ± 61.7
Ethyl Ester	96.3 ± 23.2	140.8 ± 39.8	395.8 ± 13.2
N,N-dimethylamide	97.2 ± 20.6	110.1 ± 13.3	418.5 ± 22.7

The permeation of verapamil from delivery systems containing lauryl alcohol, lauric acid propyl ester, lauric acid ethyl ester, and lauric acid N,N-dimethylamide, respectively, was therefore studied across both delipidized and stripped skin sections.

Table II summarizes the permeation rates of verapamil across the whole, delipidized and stripped skin specimens from control and delivery systems containing the respective permeation enhancers. In almost all cases, the permeation rate across the delipidized skin was higher than that across the whole skin, indicating that the stratum corneum lipids do form a barrier to the permeation of verapamil. The only exception was lauric acid N,N-dimethylamide, in which case the permeation rate across the delipidized skin was not significantly higher than that across the whole skin, implying that the protein gel in the delipidized skin still retains the barrier properties of the stratum corneum. The removal of both lipid and protein components of the stratum corneum by stripping substantially enhances the skin permeation rate of verapamil as compared to that across the delipidized skin, indicating that the protein component did have significant barrier role in the permeation of verapamil.

Table III

Enhancement of the Permeation Rate of Verapamil through Various Skin Tissues of Hairless Mouse by Lauric Acid Derivatives.

Enhancer	Enhancement Factor		
	Whole Skin	Delipidized Skin	Stripped Skin
Control	1.0	1.0	1.0
Lauryl Alcohol	1.7	1.5	1.1
Lauric Acid Derivatives			
Propyl Ester	1.8	1.9	1.2
Ethyl Ester	2.3	1.4	1.5
N,N-dimethylamide	2.3	1.1	1.9

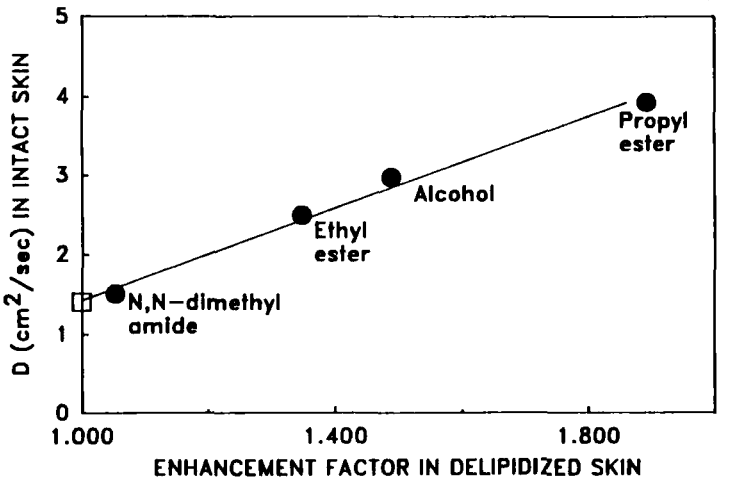

FIGURE 3

Relationship Between Diffusivity in Intact Skin and Enhancement Factor in Delipidized Skin.

Table III lists the enhancement factors for the permeation of verapamil across whole, delipidized and stripped skin, for the respective compounds. With the exception of lauric acid N,N-dimethylamide, all the compounds enhanced the permeation rate of verapamil across both whole and delipidized skin. This means that these compounds, with the exception of lauric acid N,N-dimethylamide, must have exerted their action on both the lipid and the protein components in the stratum corneum, since the stratum corneum in delipidized skin consists only of the protein gel matrix.

The data indicate that the lauric acid derivatives increased the diffusivity of verapamil in the skin. They also enhanced the permeation rate of verapamil across delipidized skin, implying some action on stratum corneum proteins. It is possible that these two phenomena are related. The data in Fig. 3 show that indeed there exists a very good linear relationship between the enhancement factor in the delipidized skin and diffusivity in the whole (intact) skin. What this relationship suggests is that the increase in diffusivity caused by these compounds is due, at least in part, to their action on the protein component of the stratum corneum.

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