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Influence of various penetration enhancers on the in vitro permeation of amino acids across hairless mouse skin

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Summary

The influence of various penetration enhancers including propylene glycol, oleic acid, Azone®, isopropyl myristate, valine, and nanoparticles on the permeation coefficient for the permeation of amino acids through hairless mouse skin as well as a dialysis membrane was assessed in vitro. The two different types of membranes were employed in order to distinguish between effects due to thermodynamic parameters and those due to barrier resistance. Furthermore, the influence of these penetration enhancers on the amount of amino acids remaining within the skin was determined. Oleic acid was found to be the most efficient enhancer for amino acids (enhancement factor (EF) of 176 for histidine) followed by Azone® (EF of 45 for phenylalanine). All other penetration enhancers failed to exert any significant effect on the skin permeation of amino acids. The fact that the enhancement effects of oleic acid and Azone® are not reversible and that the enhancers exhibited no influence with dialysis membranes clearly indicate that both penetration enhancers induce their effects on the basis of changes in skin morphology. Choosing arginine, histidine and phenylalanine as test permeants enabled a correlation between the enhancement effects and the degree of ionization of the test permeant. Histidine is the only amino acid which is unionized at pH 7.4 due to its isoelectric point. This might be the reason why the permeation enhancement induced by valine was only detectable with histidine, and not with the other two amino acids. Neither penetration enhancer resulted in any significant effect on the amount of the amino acid accumulated in the skin.

Introduction

Recent studies (Ruland and Kreuter, 1991) have demonstrated that amino acids cannot penetrate into and permeate through hairless mouse skin in significant amounts after dermal application. For this reason, absorption enhancers must be applied in order to increase the absorption of amino acids. Basically, the two principal ways in which one may optimize the bioavailability of topically applied drugs are: (1) increasing the thermodynamic activity in the vehicle (Higuchi, 1977); and/or (2) reduction of the barrier function (Allenby et al., 1969a,b: Chandrasekaran and Shaw, 1978). The latter procedure increases the rate of penetration of poorly absorbed substances by the inclusion of penetration enhancers in a

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formulation. The penetration enhancers reversibly reduce the barrier resistance of the stratum corneum and thus allow the drug to penetrate more readily to the viable tissues and enter the systemic circulation (Barry, 1983). Penetration enhancers should be safe, nontoxic, nonirritating, and pharmacologically inert while reversibly modifying the barrier properties of the skin. The question remains as to the manner in which such mild, reversible modification operates. A general theory for the action of penetration enhancers in stratum corneum, further strengthened by differential scanning calorimeteric (DSC) studies (Barry, 1987; Golden et al., 1987) involves an interaction with the lipid bilayers within the intercellular spaces. Enhancers such as dimethylsulfoxide and 1-methyl-2-pyrrolidone increase the fluidity of lipids in the skin whereas Azone® (1-dodecylazacycloheptan-2-one) and oleic acid disrupt the lipid structure. Cosolvents such as propylene glycol solvate the intracellular keratin and occupy hydrogen-bonding sites which in turn increase drug mobility. In addition, the partition coefficient for the distribution of the permeant from the vehicle to the skin, is increased by propylene glycol.

Combinations of Azone® (Wotton et al., 1985; Sheth et al., 1986) and oleic acid (Cooper, 1984; Cooper et al., 1985; Barry and Bennet, 1987) with propylene glycol lead to synergystic effects in accordance with the modes of action outlined above. Nevertheless, although the route of drug permeation through the stratum corneum has become more fully understood, the classification of penetration enhancers according to their properties, in particular, as to whether they induce polar rather than non-polar penetration, remains a difficult task.

The objective of the present paper was to perform an in vitro investigation of the effects of a number of widely accepted penetration enhancers, e.g., propylene glycol, Azone®, oleic acid and isopropyl myristate, as well as substances which have thus far not been considered as enhancers, such as nanoparticles and valine, on the permeation of amino acids. In addition, an evaluation of the mode of action was carried out. The use of an inert dialysis membrane besides hairless mouse skin allows one to distinguish between the effects due to changes in thermodynamic parameters and those due to barrier resistance. Finally, the different contents of the test permeants in the skin after pretreatment with the enhancers were determined. The permeation enhancers were selected according to the following criteria:

Propylene glycol was mainly used as a vehicle and as a cosolvent owing to its synergistic enhancing effect with Azone®, oleic acid and isopropyl myristate. Azone® was selected on the basis of previous work showing that it is more effective at enhancing penetration of hydrophilic than hydrophobic compounds (Stoughton and McClure, 1983; Sugibayashi et al., 1985; Morimoto et al., 1986). A further enhancer was found to be oleic acid. Cooper (1984) reported that oleic acid mainly promoted the percutaneous absorption of lipophilic drugs while Aungst et al. (1986) described it as acting as a promoter for non-polar as well as polar drugs. However, later investigations by Barry (1987) clearly demonstrated that oleic acid enhanced polar drug absorption particularly in combination with propylene glycol. Isopropyl myristate was chosen, since Sato et al. (1988) found it to markedly increase permeation of nicorandil although the mechanism of its action is still obscure. Investigations by Sarpotdar et al. (1988), using amino acids as penetration enhancers, showed valine to be the most effective promoter for the permeation of levonorgestrel through hairless mouse skin. Finally, rather unusual absorption promoters, namely, nanoparticles, were included in our investigations. Nanoparticles are defined as polymeric particles ranging in size from 10 to 1000 nm in which the active principle is entrapped, encapsulated and/or adsorbed. The use of nanoparticles as drug carrier systems has been extensively reviewed by Kreuter (1988). Experiments performed by Kreuter et al. (1983) showed that nanoparticles were able to considerably increase the permeability of norcholestenol through dialysis membranes. Even more interesting in the context of the present paper were the results reported by Cappel and Kreuter (1991) that showed that nanoparticles slightly enhance the penetration of a polar model permeant, methanol, through hairless mouse skin.

Materials and Methods

Chemicals

Arginine, histidine, phenylalanine, valine were purchased from Serva (Heidelberg, Germany). They were all reagent grade and were used without further purification. ^{14}C-labelled amino acids were obtained from Amersham Buchler (Braunschweig, Germany) and were used as received. Propylene glycol and oleic acid (Fluka AG, Buchs, Switzerland), Azone® (Nelson Research, Irvine, U.S.A.) and isopropyl myristate (Merck, Darmstadt, Germany) were used as received.

Buffer

Phosphate-buffered saline (PBS), pH 7.4, was used, the composition of which was as follows: NaCl, 8.0 g; KCl, 0.2 g; $Na_2HPO_4 \cdot 2H_2O$, 1.5 g; KH_2PO_4, 0.2 g; distilled water to 1000 ml. The buffer was prepared to be isoosmotic using analytical grade chemicals obtained from Merck (Darmstadt, Germany).

Nanoparticles

PMMA (polymethylmetacrylate)

Methylmethacrylate (Fluka AG, Buchs, Switzerland) was purified from polymerization inhibitors according to the methods of Riddle (1954) and Tessmar (1961) by washing 100 ml of the monomer three times with 20 ml of a solution of 5 g sodium hydroxide and 20 g sodium chloride in 100 ml distilled water. Subsequently, the monomer was washed three times with 20 ml distilled water. Poly(methylmethacrylate) nanoparticles were then produced using the method of Kreuter and Zehnder (1978): a solution of 1.0% methylmethacrylate in water was polymerised by γ-irradiation (0.5 mrad, 2.2 krad/min) using a ^{60}Co source. After lyophilization they were stored in dry form.

PBCA (polybutyl-2-cyanoacrylate)

Polybutyl-2-cyanoacrylate nanoparticles were prepared as described below. Butyl-2-cyanoacrylate (Sichel-Werke, Hannover, Germany), dextran 70 (Mol. Wt. 70000) (Fluka Biochemika), and poloxamer 188 (Erbslöh, Düsseldorf, Germany) were used as received. All other chemicals were reagent grade. 1 ml of the monomer was added dropwise to 100 ml of a stirred aqueous solution of 1% (w/v) dextran 70 and 0.2% (v/v) poloxamer 188 in 0.01 N hydrochloric acid at room temperature. In order to fully disperse the monomer, the dispersion was agitated with a glass-coated magnetic stirring bar at 1000 rpm. After 2 h, the reaction was stopped by adding 0.01 N NaOH until a pH value of 6.0 was reached, thereby avoiding the possibility of residual monomer being present. The resulting suspension was filtered through a sintered glass funnel (grade 4,

TABLE 1

Donor vehicle compositions

Vehicle	PBS solutions of					
	PG (% v/v)	OA (% v/v)	Azone (% v/v)	IPM (% v/v)	Valine (% g/v)	Nanoparticles (% g/v)
A	50	–	–	–	–	–
B	50	3	–	–	–	–
C	50	5	–	–	–	–
D	50	–	3	–	–	–
E	50	–	5	–	–	–
F	50			5		
G	50	–	–	10	–	–
H	–	–	–	–	2	–
I	–	–	–	–	–	1

PG, propylene glycol; OA, oleic acid; IPM, isopropyl myristate.

pore size 11–16 μm) and lyophilized. Prior to use, the appropriate amount of lyophilized nanoparticles was dispersed in PBS buffer.

PHCA (polyhexyl-2-cyanoacrylate)

Polyhexyl-2-cyanoacrylate nanoparticles were prepared according to the method described for PBCA nanoparticles.

Diffusion membranes

Female hairless mice, strain hr/hr-c3H/Tif Bom (Bommice Bomholtgard Breeding and Research Center Ltd, Ry Denmark) (aged 17–22 weeks) were used for the permeation studies. They were killed with CO_2. Both abdominal and dorsal skin was used after careful excision and cleaning from the subcutaneous tissue. The Medicell® dialysis membrane, pore size 24 Å, was purchased from Fischer (Frankfurt, Germany).

Preparation of test solutions

In the initial experiments, the effects of the various penetration enhancers were examined by charging the donor compartment with one of the vehicles given in Table 1.

In vitro skin permeation

Freshly excised hairless mouse skin was mounted in a two-chamber, side-by-side diffusion cell with the stratum corneum facing the donor half cell. The cell was made of glass. Both donor and receiver compartments had a volume of 1.5 ml. The surface area of the membrane of the diffusion cell was 0.8 cm^2. The cell was then immersed in a constant temperature water bath of 37 ± 1°C.

In the first set of diffusion experiments, the donor chamber was charged with one of the vehicles listed in Table 1. Such solutions contained 10 mg/ml of the tested amino acid (test permeant) and were spiked with radiolabelled amino acid (3.3 μCi/ml). The receiver compartment was filled with 1.5 ml of the phosphate buffer. The contents of the diffusion cell were allowed to equilibrate and were stirred at 100 rpm. The moment of charging the donor half cell marked the beginning of the diffusion run. Before taking a sample of the donor compartment, 4 min were allowed to elapse to ensure that a uniform distribution was achieved. This sample was used as a close estimation of the initial donor cell concentration. At specified time intervals, samples of 200 μl were withdrawn from the receiver compartment. An equivalent amount of the buffer solution (200 μl) was added to maintain a constant volume. This dilution of the receiver content was taken into account when evaluating the permeation data. The samples were transferred immediately to vials containing 7 ml scintillation cocktail (ready protein +, Beckman Instruments, München, Germany) and the amount of the radioactivity (disintegrations per min, dpm) was determined using a liquid scintillation counter (Model LS 1501, Beckman Instruments, München, Germany). After 5 h, the first set of permeation experiments was finished. The donor and receiver compartments were evacuated and rinsed three times with saline in order to remove all residual enhancers and test permeants.

A second set of diffusion experiments was initiated with the same skin patches still mounted in the diffusion cells. The permeation procedure was the same as that described above with the exception of the donor charge. Here, the content of the donor chamber constituted phosphate buffer and the appropriate volume of the respective amino acid. This second set of experiments is referred to as the 'reversibility experiment'. If the dialysis membrane was mounted between the diffusion cells, the above outlined permeation procedure was used. This time samples of 50 μl were taken every 5 min for only 1 h in order to maintain sink conditions.

The data analysis was described in detail previously (Flynn et al., 1974; Durrheim et al., 1980; Ruland and Kreuter, 1991).

Skin reservoir

This study was designed to allow a further estimation of a possible skin reservoir of amino acids induced by the pretreatment with penetration enhancers. Therefore, the skin samples from each run were analysed. After the end of each experiment, the skin patches were removed care-

fully from the diffusion cells and immersed in distilled water. After several washings, the skin patches were dried with pieces of cellulose and placed into glass vials containing 1 ml of the tissue solubilizer BTS-450 (Beckman Instruments, München, Germany). The samples were kept at 60°C until they dissolved completely. The samples were decolorized with 50 μl of 30% H_2O_2 (Merck Darmstadt, Germany). Then, 70 μl neat acetic acid (Merck, Darmstadt, Germany) were added to eliminate chemiluminescense. After adding 10 ml ready organic cocktail (Beckman Instruments, München, Germany), the amount of radioactivity was determined using the same scintillation counter as mentioned above.

Results

As mentioned previously, our present study was carried out to investigate the effect and, as far as possible, the mode of action of a number of penetration enhancers on the permeability of amino acids as well as their capacity to build up a drug reservoir in the skin. Therefore, arginine, histidine, and phenylalanine were chosen as test permeants, since they provide a representative cross-section of all amino acids with reference to their degree of ionization (Ruland and Kreuter, 1991). At pH 7.4, histidine is neutral according to its isoelectric point whereas arginine is positively charged and phenylalanine negatively charged. Consequently, by choosing these three amino acids we were able to investigate an eventual relationship between enhancing effects and the degree of ionization of the test permeants. The concentrations of the various enhancers were selected by using the lowest possible concentration that exhibits a significant enhancement effect within a few hours according to the literature.

The influence of the various penetration enhancers on the permeation of arginine through hairless mouse skin and the dialysis membrane, respectively, is shown in Fig. 1a and b. Oleic acid is the most efficient penetration enhancer, followed by Azone®. All other penetration enhancers have no significant effect on the permeation of arginine. An increase in oleic acid concentration does not influence the permeability coefficient in the concentration range investigated whereas Azone® increases the coefficient with increasing concentration. These effects are not reversible. In the case of Azone® even an additional slight increase in permeability was observed in the reversibility experiment (see Materials and Methods). In the studies in which the skin

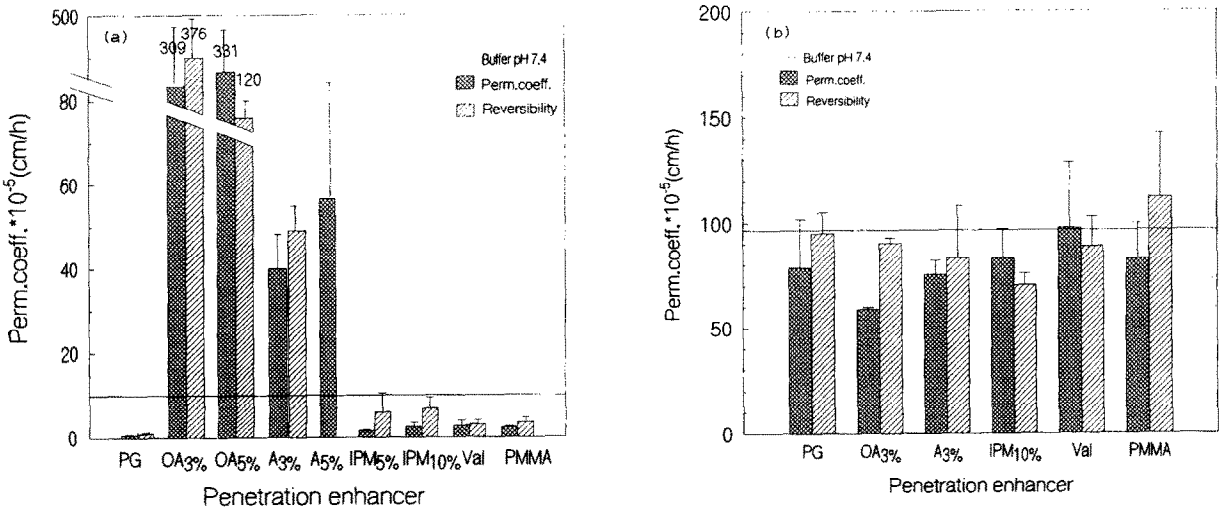

Fig. 1. Effect of different penetration enhancers on the permeation of arginine through hairless mouse skin (a) or dialysis membrane (b), respectively. All data represent the means of three experiments ± SD. The straight line depicts the permeability coefficient of the test permeants when phosphate buffer constitutes the medium in the donor compartment.

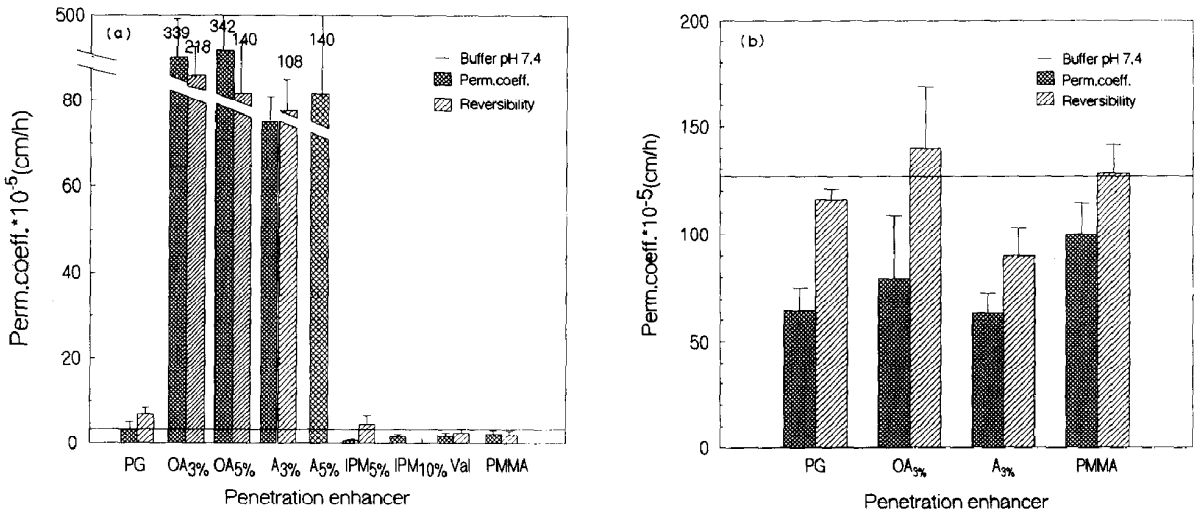

Fig. 2. Effect of different penetration enhancers on the permeation of phenylalanine through hairless mouse skin (a) or dialysis membrane (b), respectively. All data represent the means of three experiments ± SD. The straight line depicts the permeability coefficient of the test permeants when phosphate buffer constitutes the medium in the donor compartment.

was substituted by dialysis membranes, the penetration enhancers have no effect or even a decreasing influence on the permeability of arginine. These results clearly demonstrate that oleic acid and Azone® do not increase the thermodynamic activity of the amino acid but rather exhibit their effects on the basis of changes in barrier resistance. The decreasing penetration coefficients through dialysis membranes (Fig. 1b) may be attributed to a decrease in the thermodynamic activity of arginine in the more lipophilic vehicles because substitution of the donor by enhancer-free buffer in the reversibility experiment shows reversible effects with this type of membrane. Similar permeation patterns through hairless mouse skin and dialysis membrane were

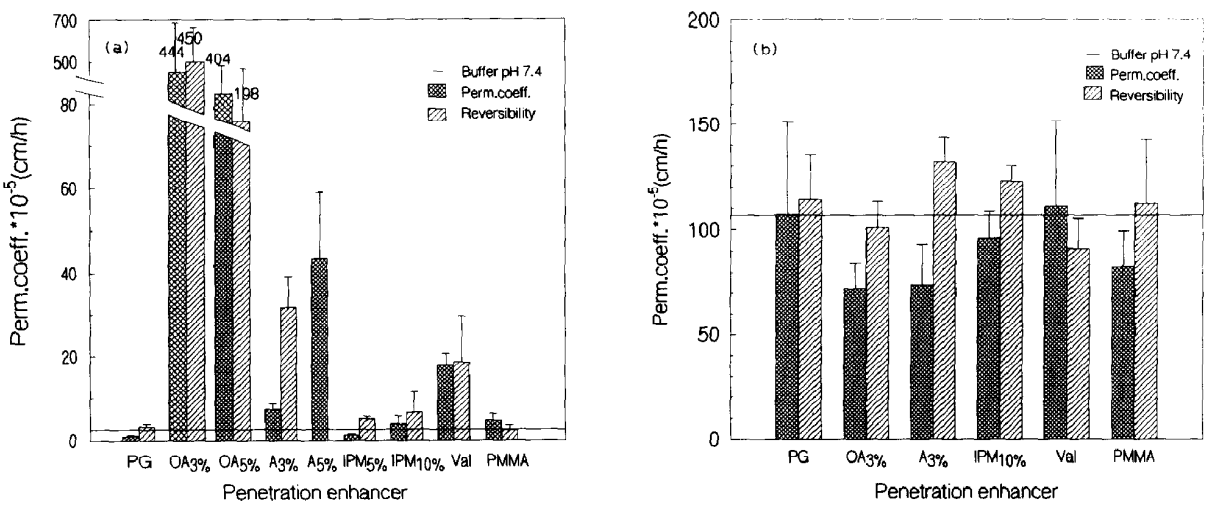

Fig. 3. Effect of different penetration enhancers on the permeation of histidine through hairless mouse skin (a) or dialysis membrane (b), respectively. All data represent the means of three experiments ± SD. The straight line depicts the permeability coefficient of the test permeants when phosphate buffer constitutes the medium in the donor compartment.

TABLE 2

Enhancement factors (EF) of the respective penetration enhancers at different concentrations using hairless mouse skin as a membrane

Penetration enhancer (concentration (%))	Arginine		Histidine		Phenylalanine	
	EF ± SD	*P*	*EF* ± SD	*P*	*EF* ± SD	*P*
PG (50%)	0.05 ± 0.01	hs	0.42 ± 0.06	s	1.03 ± 0.51	ns
OA (3%)	29.45 ± 5.20	hs	176.35 ± 27.29	hs	111.25 ± 19.41	hs
OA (5%)	31.84 ± 4.50	hs	176.64 ± 26.26	hs	113.38 ± 6.91	hs
A (3%)	4.13 ± 0.61	hs	3.10 ± 0.35	hs	25.00 ± 0.36	hs
A (5%)	5.32 ± 1.24	hs	17.82 ± 0.82	hs	45.15 ± 16.72	hs
IPM (5%)	0.13 ± 0.08	hs	0.48 ± 0.04	s	0.23 ± 0.08	hs
IPM (10%)	0.23 ± 0.04	hs	1.45 ± 0.38	ns	0.50 ± 0.10	hs
Val (2%)	0.24 ± 0.09	hs	7.84 ± 1.33	hs	0.60 ± 0.15	s
PMMA (1%)	0.23 ± 0.02	hs	1.91 ± 0.05	ns	0.70 ± 0.27	ns

The enhancement factor is defined as the ratio of permeability coefficient with penetration enhancer/permeability coefficient without penetration enhancer. ns, not significant, s, significant ($p \ll 0.05$); hs, highly significant ($p \ll 0.01$). PG, propylene glycol; OA, oleic acid; A, Azone®, IPM, isopropyl myristate; Val, valine; PMMA, poly(methylmethacrylate) nanoparticles.

observable with phenylalanine (Fig. 2a and b) except for the enhancing effect of Azone®. Azone® enhanced the permeation of phenylalanine to a much higher extent than that of the other two amino acids.

Histidine shows a slightly different permeation pattern (Fig. 3a and b). Here again, oleic acid is the most efficient penetration enhancer. Oleic acid enhanced the permeation of histidine to a higher extent than the permeation of arginine and phenylalanine. Azone® is even less effective than with arginine. Whether these findings correlate with the neutral ionization behavior of histidine at the experimental pH is not yet certain.

Histidine is the only amino acid whose permeability is slightly increased by valine (factor 7). The valine effect is not reversible and does not occur with the dialysis membrane. Detailed information about the findings of the investigated penetration enhancers on the penetration of arginine, histidine and phenylalanine are given in Table 2 by evaluating the enhancement factors

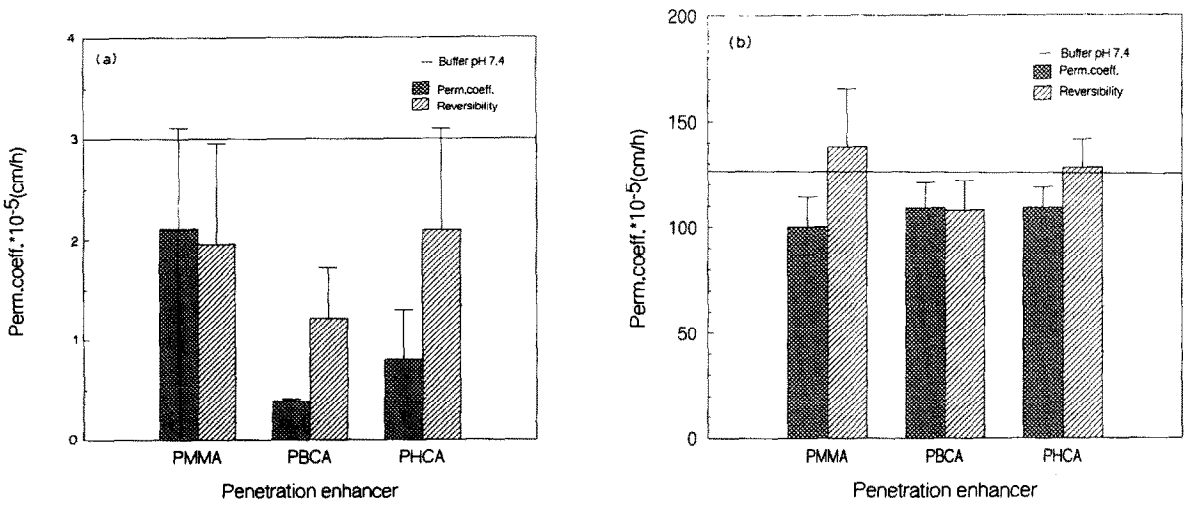

Fig. 4. Effect of different types of nanoparticles on the permeation of phenylalanine through hairless mouse skin or dialysis membrane, respectively. All data represent the means of three experiments ± SD. The straight line depicts the permeability coefficient of the test permeants when phosphate buffer constitutes the medium in the donor compartment.

(the enhancement factor (EF) is defined as the ratio of permeability coefficient with penetration enhancer/permeability coefficient without penetration enhancer). The relatively low enhancement factors of arginine may be explained with regard to the rather high intrinsic permeation coefficient of arginine (10×10^{-5} cm/h) without using a penetration enhancer in comparison to the lower values for histidine and phenylalanine ($2\text{–}3 \times 10^{-5}$ cm/h) (Ruland and Kreuter, 1991).

Fig. 4 depicts the influence of different types of nanoparticles on the permeation of phenylalanine through hairless mouse skin and dialysis membrane, respectively. The permeation of phenylalanine is either unaffected by nanoparticles or decreases as a function of nanoparticle hydrophilicity. PBCA nanoparticles, the most hydrophilic particles, result in the highest permeability decrease. PMMA nanoparticles are relatively lipophilic and appear to have the lowest decreasing influence on the permeation of phenylalanine. For this reason, they were further investigated with the other amino acids. However, no enhancing effect was observable.

The amount of amino acid in the skin is either unaffected or only slightly increased by the tested penetration enhancers (Table 3). The amount of amino acids in the skin slightly increases with increased permeability of the respective amino acid caused by enhancer addition. However, a considerable accumulation of amino acids in the skin is not observed. Even by using penetration enhancers, the total amount of amino acids in the skin is only 0.4% of the initial amount in the donor compartment.

Discussion

As shown in Results, only oleic acid and Azone® exhibited significant amino acid permeation enhancing effects in the skin whereas valine showed a modest enhancement only on the permeation of histidine.

In our studies, propylene glycol showed no penetration promoting effect on the amino acids tested although it was previously found to act as a penetration enhancer, on the basis of its keratin solubilizing effects and or its effects on promoting drug partitioning into the skin. However, the investigations of Barry and Bennett (1987) also revealed similar negative effects for propylene glycol on the permeation of mannitol, hydrocortisone and progesterone through human skin.

Considering the three mechanisms proposed by Aungst et al. (1990), by which fatty acids might

TABLE 3

Influence of the penetration enhancers of the amount of amino acid in the skin

Penetration enhancer (%)	Arginine			Histidine			Phenylalanine		
	Amount in skin (%)	F	P	Amount in skin (%)	F	P	Amount in skin (%)	F	P
Control	0.09 ± 0.05			0.07 ± 0.06			0.13 ± 0.03		
PG	0.05 ± 0.02	0.5	ns	0.08 ± 0.02	1.1	ns	0.06 ± 0.00	0.5	s
OA (3%)	0.37 ± 0.09	4.1	hs	0.63 ± 0.05	9.0	hs	0.53 ± 0.06	4.1	hs
OA (5%)	0.35 ± 0.04	3.8	hs	0.46 ± 0.13	6.6	hs	0.42 ± 0.07	3.2	hs
A (3%)	0.11 ± 0.07	1.2	ns	0.08 ± 0.05	1.1	ns	0.10 ± 0.07	0.8	ns
A (5%)	0.29 ± 0.05	3.2	hs	0.27 ± 0.13	3.9	hs	0.29 ± 0.05	2.2	hs
PMMA	0.01 ± 0.00	0.1	ns	0.01 ± 0.00	0.1	ns	0.02 ± 0.00	0.2	hs
IPM (5%)	0.10 ± 0.04	1.1	ns	0.20 ± 0.07	2.9	ns	0.09 ± 0.01	0.7	ns
IPM (10%)	0.02 ± 0.02	0.2	ns	0.03 ± 0.01	0.4	ns	0.04 ± 0.00	0.3	hs

Mean (± SD) amounts in % of the total radioactivity of the amino acid determined in the respective skin patches after each sequential run. The initial activity for all amino acids was normalized to 10^7 dpm. F is a factor which is defined as the ratio of amount (%) in skin with/without penetration enhancer. ns, not significant; s, significant ($p \ll 0.05$); hs, highly significant ($p \ll 0.01$). PG, propylene glycol; OA, oleic acid; A, Azone; IPM, isopropyl myristate; PMMA, poly(methylmethacrylate) nanoparticles.

increase skin permeation rates, i.e., (i) disruption of the lipid regions of the stratum corneum; (ii) increased skin/vehicle partitioning by ion pair formation; and (iii) increasing solvent drag mechanism only the first one is in accordance with our results. Ion pair formation with the carboxylate anion of the fatty acid as assumed by Green and Hadgraft (1987) in the case of the transport of cationic β-blocking agents seems unlikely to have occurred in our permeability studies. If ion pair formation does play a major role in our case the cationic arginine should reveal a stronger effect in comparison to the other amino acids which are not positively charged. In addition, ion pair formation should also be observable in the dialysis membrane studies. The proposed solvent drag mechanism of Yamada et al. (1987) for the oleic acid induced enhancement of molsidomine skin penetration is unlikely in our case, since such effects should have been reversible. At present, it seems most likely that oleic acid exerts its penetration promoting effects on the basis of lipid perturbation. In this context, Barry (1987) assumed the following mechanism of increased lipid fluidity. Due to the great similarity with stratum corneum lipids, oleic acid is likely to partition into this barrier domain. Once incorporated, the 'kinked' structure (arising from the *cis* double bond) of oleic acid will disrupt and increase the fluidity of the lipid packing, and hence, decrease the diffusional resistance to permeants. In contrast, Francoeur et al. (1990) and Ongpipattanakul et al. (1991) proposed a different mechanism. Substantiated by X-ray diffraction and Fourier transformed infrared spectroscopic studies, they assumed the existence of separate liquid and solid phases within oleic acid-treated stratum corneum. As a consequence, the enhanced transport of polar compounds across the stratum corneum can be explained by the formation of permeable interfacial 'defects' within the stratum corneum lipid bilayers which effectively decrease either the diffusional path length or the barrier resistance. Our findings correlate very well with both suggestions as these mechanisms are not reversible within 2 h and do not work with inert membranes.

A similar mechanism was discussed for Azone® by Goodman and Barry (1989) and Beastall et al. (1988). Azone® is a nonpolar molecule which partitions directly into the lipid regions of the tissue. The Azone® inserted in the skin prevents the lipid chains from associating and crystallizing. According to this mechanism, reversibility and effects on inert membranes cannot be expected. Within this context, Wotton et al. (1985) showed by multidose experiments that a single application of Azone® is capable of enhancing the permeation of subsequent doses of drugs for a period of several days. This correlates very well with our observation of irreversible effects of Azone®. In addition, a concentration-dependent enhancement by Azone® on the penetration of triamcinolone acetonide through hairless mouse skin was previously shown by Chow et al. (1984). However, a quantitative comparison of the effects of Azone® with other test permeants is not meaningful because the Azone® effects are strongly dependent on the vehicle, cosolvent, test permeant, and diffusion membrane.

Moreover, it also should be mentioned that such strong effects as indicated by our results for the oleic acid induced enhancement of amino acid permeation through hairless mouse skin may not occur to the same extent in human skin. Bond and Barry (1988) clearly demonstrated in their investigation of 5-fluorouracil through human and hairless mouse skin that hairless mouse skin exhibited exaggerated responses to the penetration enhancer formulations.

Isopropyl myristate has no influence or even a permeability reducing effect. This contrasts with the study of Sato et al. (1988) who reported a dramatic increase in nicorandil penetration through hairless rat skin with this compound. One could argue that the experimental time of 5 h would be too short for isopropyl myristate to exhibit any effects. However, a long time study with phenylalanine (10% isopropyl myristate in propylene glycol) through hairless mouse skin over 12 h clearly demonstrates that even within this time frame no effects are observable with this compound (Fig. 5).

As mentioned in the Introduction, valine was tested as a penetration enhancer in the expectation of confirming the findings of Sarpotdar et al.

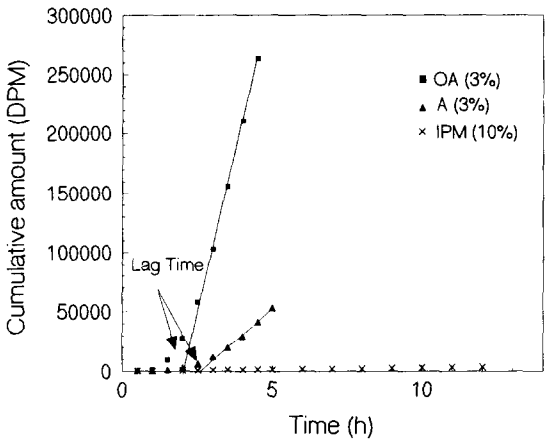

Fig. 5. Representative penetration profile for phenylalanine diffusing through full thickness hairless mouse skin.

(1988). These authors investigated 15 amino acids and found them to enhance the rate of penetration of levonorgestrel through hairless mouse skin. Valine showed the greatest effects by enhancing the penetration rate of levonorgestrel by a factor of 10. In our study, valine was not effective with arginine and phenylalanine but did enhance the penetration of histidine by a factor of 7. However, it is difficult to determine the mode of action of valine in combination with histidine. One possible explanation may be a correlation with the degree of ionization of the test permeant since histidine is the only amino acid used which is unionized.

The largely irreversible decrease in permeability coefficient through hairless mouse skin but not through dialysis membranes with nanoparticles is difficult to explain (Fig. 4a and b). Moreover, the decrease in permeability increases with hydrophilicity of the polymer. A very hypothetical explanation for this phenomenon could be that the more hydrophilic polybutylcyanoacrylates have greater bioadhesiveness and therefore, adhere to the skin to a higher extent (Kreuter, 1990). The adhering nanoparticles possibly were not removed by the washing procedure prior to the reversibility experiment. In both cases, in the first sequential run as well as in the reversibility experiment, these adhering nanoparticles may have acted as an additional permeation barrier. A similar phenomenon has been observed with liposomes (Bouwstra, J., personal communication). The results indicate that if this bioadhesion hypothesis holds true, the (bio)adhesion of the nanoparticles to the skin would be much greater than to the dialysis membrane.

In conclusion, these investigations have confirmed that oleic acid and Azone® are potential candidates for enhancing the permeation of amino acids through hairless mouse skin. In accordance with their proposed mode of action as stratum corneum lipid region perturbants, none of the demonstrated enhancement effects are reversible within a couple of hours. Neither of the investigated penetration enhancers has a significant influence on the amount of amino acid accumulation in the skin.

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