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Evaluation of drug penetration into human skin ex vivo using branched fatty acids and propylene glycol

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Abstract

The influence of two middle chain methylbranched fatty acids and propylene glycol on the penetration of the highly lipophilic model substance pyrene butyric acid into human skin ex vivo has been investigated. The results obtained were compared with experimental data using oleic acid as standard enhancer. Analogously, liberation studies of pyrene butyric acid into artificial lipid acceptor membranes were performed to evaluate the contribution of vehicle effects to the skin penetration results. It was shown that the fatty acids initially improve the liberation of the model substance which corresponds to an increase in skin penetration. When the penetration process was assessed, the dermal concentration profiles of pyrene butyric acid and propylene glycol were almost the same, strongly indicating a cotransport for the lipophilic model substance. Levels of both pyrene butyric acid and propylene glycol did increase when the vehicles contained fatty acid. Furthermore, there is some indication of a more specific action of oleic acid within the stratum corneum. However, the major effect on the penetration of pyrene butyric acid arises from propylene glycol. It is caused by solvent properties and solvent drag or favoured partition, respectively, into the stratum corneum and the hydrophilic epidermis and dermis which are supposed to be the main diffusion barrier for the model penetrant. Copyright © 1996 Elsevier Science B.V.

Keywords: Branched fatty acids; Cosolvent; Liberation; Lipophilic penetrant; Membrane; Propylene glycol; Skin penetration

1. Introduction

In recent years, interest has been focused on the determination of the influence of chemical substances on both dermal and transdermal drug transport. A large variety of different agents has

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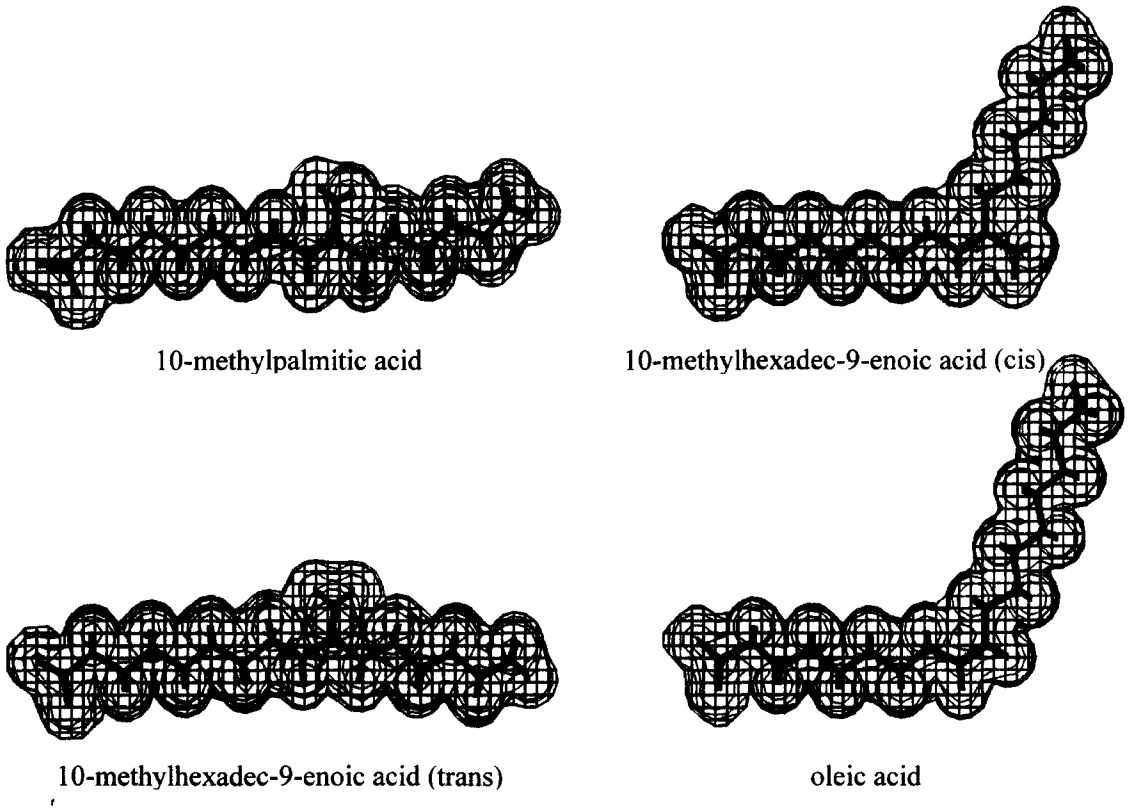

Fig. 1. Chemical structures of the fatty acids.

been examined and there has been a large number of investigations into their mode of action. Additionally, much effort has been made to find out reasonable correlations between physicochemical and structural properties of such substances (Hori et al., 1989) and their effects on drug transport as well as on the diffusion barrier, the stratum corneum (Scheuplein and Blank, 1971).

From this point of view, fatty acids have extensively been studied, particulary oleic acid (OA) (Cooper, 1984; Cooper et al., 1985; Aungst et al., 1986; Golden et al., 1987; Green et al., 1988; Mahjour et al., 1989). It is assumed that these substances either increase the degree of disorder of the intercellular lipid hydrocarbon chains or form phase-separated fluid domains within the liquid-crystalline lipid structures reversibly causing a loss of diffusion resistance within the barrier (Mak et al., 1990; Potts et al., 1991). While the effectiveness of *cis*-unsaturated fatty acids on the highly ordered, lamellar organized stratum corneum lipids has doubtlessly been demonstrated, little effort has been made to investigate the influence of branched fatty acids.

This work examines the influence of 10-methylpalmitic acid (MP) (middle chain branched) and 10-methylhexadec-9-enoic acid (MH) (middle chain branched and double bond) (Fig. 1) on the dermal penetration of the coapplied model substance pyrene butyric acid (PBA) into human skin under ex vivo conditions. According to the literature, middle chain methylbranched fatty acids with physiological chain length are worth investigating from both toxicity and fluidity aspects (Weitzel et al., 1950; Weitzel et al., 1951). PBA was used focusing the interest at first on the extremely lipophilic properties of a model penetrant as well as for analytical reasons.

Propylene glycol (PG) was used as solvent for the model substance and the fatty acids as poten-

tial penetration modulators. It is known as an appropriate vehicle for enhancement studies with hydrophilic and lipophilic drugs (Cooper et al., 1985; Barry and Bennett, 1987; Goodman and Barry, 1989; Mahjour et al., 1989). Sufficient evidence exists that systems consisting of PG and fatty acids are more effective enhancers as compared to either PG or fatty acid alone (Cooper, 1984; Cooper et al., 1985; Aungst et al., 1986; Barry, 1987; Komata et al., 1992).

Therefore, the systems described were chosen for evaluating the effects of PG and branched fatty acids in comparison to oleic acid. Correlating the results obtained from skin penetration as well as from liberation into lipophilic partition membranes without any anisotropic properties as found for stratum corneum lipids, it should be possible to explain the contribution of drug solubilization/partitioning and solvent penetration to the interaction of PG and the fatty acids with the barrier structures.

2. Materials and methods

2.1. Chemicals

Pyrene butyric acid (PBA), (Molecular Probes, Eugene, USA), oleic acid (OA), (Fluka Chemie AG, Buchs, Switzerland), propylene glycol (PG), (Schuchardt GmbH, München, Germany) and sodium carboxymethylcellulose (SCMC) were obtained commercially and used as received for the preparation of the model formulations.

10-methylhexadec-9-enoic acid (MH), (98.2%) was synthesized by Wittig reaction starting from 8-carboxyoctyl triphenylphosphonium bromide and octan-2-one (Dobner et al., 1987). Hydrogenation of the unsaturated fatty acid on Pd/C yields quantitatively the 10-methylpalmitic acid (MP), (99.0%). The identity and purity of both fatty acids were verified by mass spectrometry and HPLC.

1-Dodecanol (DD) (Merck, Darmstadt, Germany), collodion 4% (w/w) (Caesar and Loretz GmbH, Hilden, Germany), ether (Fluka Chemie AG, Buchs, Switzerland) and ethanol (Merck, Darmstadt, Germany) were necessary to produce the lipid acceptor membranes for the in vitro liberation studies.

The determination of the partition coefficients was performed with octanol (Fluka Chemie AG, Buchs, Switzerland) as well as with isopropyl myristate (IPM), (Fluka Chemie AG, Buchs, Switzerland) as organic phases.

The gaschromatographic quantification of PG in skin samples required the addition of trimethyl sulfonium hydroxide (TMSH, Macherey Nagel GmbH and Co. KG, Düren, Germany) to improve fatty acid evaporation.

The solvents for the analytical procedures were of chromatography gradient grade and purchased from Merck (Darmstadt, Germany).

2.2. Methods

2.2.1. Solubility

The solubilities of PBA were determined in water, pure PG, PG containing 5% fatty acid as well as in pure OA. An excess of model substance was added to 3 ml of the liquids in glass ampoules. The mixtures were vigorously shaken in a water bath at 32 ± 1°C for 24 h. After centrifugation (3000 rpm) of the equilibrated samples, the PBA content of the supernatant solutions or suitable ethanolic dilutions were assayed by HPLC.

2.2.2. Partition coefficients

Partition coefficients of the model substance were examined between octanol-water, IPM-water and IPM-PG. For that purpose, the penetrant was maximally dissolved in the octanol or IPM saturated anorganic phase. 9 ml of this solution were mixed with 1 ml octanol or IPM, saturated with the anorganic phase and shaken to equilibrate at 32 ± 1°C for 24 h. After separating the two phases, each was analysed for penetrant concentration by HPLC.

The partition coefficient P was calculated from the following equation:

$$P = \frac{C_{\text{organic}}}{C_{\text{anorganic}}}$$

2.2.3. Preparation of model formulations

The basic vehicle was prepared by adding PG to PBA. The latter was used in a concentration of 0.5% (w/w) to ensure suspension-type conditions. Formulations containing fatty acid were obtained by subsequent addition of 5% (w/w) fatty acid to PG. The vehicles were prepared 1 day in advance and stored tightly shut at room temperature.

2.2.4. Ex vivo penetration studies

2.2.4.1. Preparation of the skin. Excised human skin was taken from breast skin of female patients aged 17–47 years who were undergoing a reduction plasty. In the preparation of the skin, the subcutaneous tissue was removed mechanically and then punch biopsies were carried out to obtain sheets of a constant surface area (3.14 cm^2) available for diffusion. Skin samples were wrapped in aluminium foil and storred at $-20°C$ for a short time.

2.2.4.2. Experimental procedure. The experiments were carried out in triplicate using skin samples from three different donors under in vivo mimic conditions using glass diffusion cells according to Franz (1975) (Crown Glass Company, Sommerville (New Jersey) USA.). Water was chosen as acceptor medium at $32 \pm 1°C$. No sink conditions were required because dermal not transdermal effects were studied. Our major question was whether the potential modulators influence the stratum corneum barrier. In this way epidermis and dermis already act as acceptor compartments.

20 μl of the liquid formulations were applied. After several intervals of time (30, 100, 300, and 1000 min), the skin was worked off. First, the horny layer of the treated area was removed using 20 adhesive tape strips. Then microsectioning of the viable skin was performed using a kryotome (Gefriermikrotom Modell 1205 and Frigomobil®, R. Jung, Heidelberg, Germany). After an extraction procedure with methanol the amount of recovered substances were determined with HPLC as well as with GC-MS.

2.2.5. In vitro liberation studies

2.2.5.1. Preparation of the acceptor membranes. DD, the acceptor membrane lipid, was dissolved in a mixture of ether and ethanol (85:15 v/v) and added to the same volume of collodion 4% (w/w). Thin films were drawn with an appropriate apparatus to produce a membrane after solvent evaporation. Then, it was punched out for round sheets of a constant area (12.5 cm^2). After a 2 week period of drying storage in a desiccator with silica gel, they were appropriate for liberation studies.

2.2.5.2. Experimental procedure. The multilayer membrane system (Neubert et al., 1991) was used for the liberation studies. The number of adjacent DD membranes per penetration cell depends on the solubility of the penetrant in DD. In this way sink conditions were realized. Accordingly, four DD membranes per cell were set in place to perform PBA liberation studies. The liquid formulations (10 μl) were put on the defined application area (4 cm^2) of the acceptor membrane system. The prepared penetration cells were fixed in the model construction and equilibrated at $32 \pm 1°C$. After several intervals of time (10, 30, 60, 100, and 300 min), the cells were separated and the membranes were analysed for PBA content.

2.2.6. Analytical procedure

2.2.6.1. Model substance. The concentration of PBA in the skin samples was determined by HPLC (Merck-Hitachi-system, Darmstadt, Germany) equipped with a pump (L-6200 A), a fluorescence detector (F-1080, $\lambda_{ex} = 341$ nm, $\lambda_{em} = 375$ nm), an automated sample injector (AS-4000) and an integration package (HPLC-manager, version 2) at a flow rate of 1 ml/min.

The analyses were performed with an analytical reversed phase column (LiChrospher®100, RP 18, 5 μm, 125 – 4, Merck, Darmstadt, Germany). The mobile phase was composed of 75:25:0.5 (v/v/v) acetonitrile: water: glacial acetic acid (pH 4.0).

Table 1
Influence of the fatty acids and PG on solubility and partition coefficients of PBA

Fatty acids	Without	5% MP	5% OA	5% MH
Solubility (μg/ml)				
Water	0.97 ± 0.07		Phase-separation	
PG	2640.10 ± 149.30	3408.58 ± 280.05	3512.53 ± 42.54	3397.39 ± 268.56
Partition coefficient lg P				
Octanol-water	4.3 ± 0.29		Phase-mixing (octanol-fatty acids)	
IPM[a]-water	1.7 ± 0.09		Phase-mixing (IPM-fatty acids)	
IPM-PG	−0.2 ± 0.02		Phase-mixing (IPM-fatty acids)	

Data were determined at 32 ± 1°C, each value represents the mean ± S.D., $n = 5$.
[a]IPM = isopropyl myristate.

External calibration via peak area measurements were used to determine the amount of the model substance in each sample. The analytical method was reproducible with a mean relative standard deviation of 1.5%. The detection limit for PBA was 5 ng/ml.

2.2.6.2. Propylene glycol. The solvent was determined using a GC-MS (model Finnigan MAT Magnum, GC-Varian 3400, autosampler Finnigan MAT A 200 S, Bremen, Germany). Free fatty acids as components of the skin extracts (100 μl) were transmethylated with 1 μl 0.2M TMSH in methanol to improve their evaporation. The samples were separated using a capillary column (FFAP, 25 m × 0.25 mm ID, 0.25 μm film, Macherey Nagel, Düren, Germany). The column temperature was programmed from 60°C for 1 min to 160°C with a rate of 15°C/min. The system was kept isothermal for 1 min, further on heated to 240°C with a rate of 25°C/min and kept again for 15 min. (Carrier gas helium, prepressure 13 psi, flow rate 40 cm/min, injector temperature 240°C, splittless injector, split valve opening after 1 min, chemical ionisation using methane).

PG was quantified by external peak calibration. The GC-method was reproducible with a mean relative standard deviation of 8.5%. The detection limit was 5 ng/ml.

2.2.7. Analysis of data

The results of the ex vivo penetrations and in vitro liberations are presented as mean values with standard deviation for triple and 5-fold determinations, respectively. All data were evaluated using the one way analysis of variance (ANOVA) with the all pairwise multiple comparison procedure according to the Student-Newman-Keuls method. For analysing the solubility data and partition coefficients 5-fold samples from each experiment were used. The one way ANOVA was also performed for comparing the results. In either investigation, a level of significance of 95% was considered to be different.

3. Results and discussion

3.1. Physicochemical data of PBA

PBA is sparingly soluble in water (Table 1). The solubility increases about 2500 fold using PG. The addition of the fatty acids leads to a further increase in solubility as compared to the base vehicle. There are no significant differences between the individual fatty acids.

The partition coefficient of PBA (octanol-water, lg P = 4.3) confirms the high lipophilicity of the model substance. Octanol and water were used to obtain comparable information about the lipophilicity of the penetrant. The miscibility of octanol and PG unables one to study the influence of PG on the partition coefficient. Therefore, further determinations were carried out using IPM as organic phase. According to Poulsen et al. (1968) and Hadgraft and Ridout (1987), IPM corresponds to the physicochemical properties of the stratum corneum as well as octanol. Phase mixing

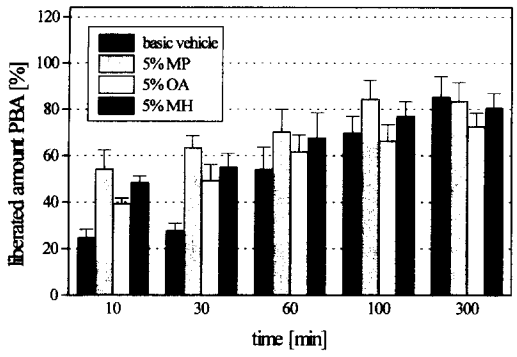

Fig. 2. Influence of the fatty acids on the liberation of PBA into the multilayer membrane system, (Each point represents the mean ± S.D., $n = 5$).

is the reason that the influence of the fatty acids on the partition coefficient with IPM was not determined.

3.2. Liberation of PBA

The results summarized in Fig. 2 indicate high PBA release rates from PG with significant differences between the fatty acid containing vehicles and the reference up to 30 min. A difference between the individual fatty acids could only be observed for 10 min, when a slight preference for the middle chain methylbranched compounds is detectable. Based on the assumption that the in vitro model qualitatively reflects changes in the vehicle release, one can establish that initially the fatty acids improve PBA liberation. This occurs in accordance with its solubility increase compared to PG. From the thermodynamical point of view, in spite of solubility differences in the formulations, equal activity conditions for PBA exist at $t = 0$ min because of the use of suspension-typ systems (Davis and Hadgraft, 1991). Afterwards, in maintaining the saturation solubility of PBA and therefore, maximal thermodynamic activity within the donor, differences could arise due to the addition of the fatty acids.

Considering the time aspect, differences are compensated at about 60 min. Whether it is caused by thermodynamic reasons or by the levelled influence of the artificial model membranes remains unclear. Possibly, both effects are jointly responsible.

3.3. Penetration of PBA

3.3.1. Stratum corneum

Using fatty acids, the liberation results are reflected by significant increased PBA concentrations within the stratum corneum at the initial time (Fig. 3a). Whereas from the basic vehicle slightly increasing amounts of PBA penetrate up to 300 min, a continuous decrease is obvious from the formulations containing MP and MH leading to a concentration adjustment at this time. The lowered levels correlate with the uptake of PBA into the viable skin layers (Fig. 5a) and indicate

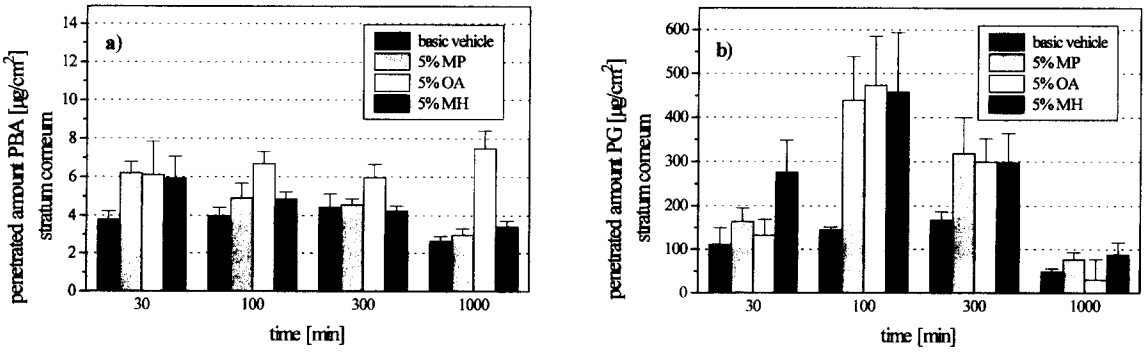

Fig. 3. (a) Influence of the fatty acids on the penetration of PBA into the stratum corneum, (Each point represents the mean ± S.D., $n = 3$). (b) Influence of the fatty acids on the penetration of PG into the stratum corneum, (Each point represents the mean ± S.D., $n = 3$).

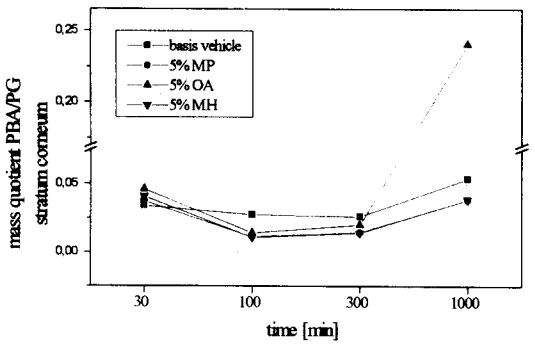

Fig. 4. Mass ratio of PBA and PG within the stratum corneum at different intervals of time.

donor limited penetration, although suspension-type conditions should still be maintained in all vehicles. In the same way, a reduction occurs for the reference but later, i.e. between 300 and 1000 min.

In contrast, addition of OA results in a constant level of PBA until the end of the experiment. The reason remains speculative, but should be aside from thermodynamic differences because of similar conditions. A possible explanation could proceed from the finding that OA participates in the formation of distinct heterogeneous fluid phases within the gel or solid phases of the endogeneous stratum corneum lipids (Potts and Francoeur, 1993). Based on the knowledge of the PBA-solubility in OA (4.9 ± 0.2 mg/ml), one can assume that such phases, additionally to the bilayer disrupting properties act as solvent pool storing PBA. According to IR-spectroscopic investigations, the action of OA is known to exceed its application-time (Mak et al., 1990) what possibly give reason for the long term effect of this fatty acid. In any event, using OA, the PBA penetration is affected by other or additional influences compared to the reference and the formulations containing MP and MH.

The comparison of Fig. 3a and 3b reveals similar stratum corneum profiles for PBA and PG from the basic vehicle. Evaluating quantitatively the mass transfer of both substances by calculating the quotient $m_{\text{PBA/Stratum corneum}}/m_{\text{PG/Stratum corneum}}$, the ratio of PBA to PG can be shown to be constant up to 300 min (Fig. 4). This suggests that PBA and PG penetrate simultaneously into the stratum corneum. The ratio is increased at 1000 min indicating an accumulation or deposition of PBA due to the more rapid PG-transfer into the viable epidermis.

With use of the fatty acids the ratios calculated were somewhat different from the basic vehicle in such a way that the quotients are decreasing up to 100 min, but increasing as well up to 1000 min. On the one hand, the decrease in PBA level contributes to this result. It corresponds with the liberation findings in so far as the maintenance of the maximal thermodynamic activity within the donor is possibly not guaranteed. On the other hand, PG itself penetrates even better when the fatty acids are added (Fig. 3b), thus reducing the ratio. The marked rise in the mass quotient using OA is caused by the accumulation of PBA. It cannot be correlated with the amount of PG.

3.3.2. Viable skin

Concerning the penetrated PBA amount of the viable part of the epidermis and dermis, a time dependent accumulation occurs for all formulations due to the substance influx but lacking sink conditions of the acceptor liquid (Fig. 5a). However, there are quantitative differences between the basic and the other vehicles. A significant augmentation of PBA levels using the formulations containing fatty acid is evident after an initial time. To some extent, the improved liberation will be contributing to this result. But another influence seems to be more pronounced. From Fig. 5a and b it appears that the concentration profiles of PBA and PG are almost the same, strongly indicating a transport of the model substance associated with PG. Considering the fact of the poor solubility of PBA in aqueous mediums, the substance obviously passes the hydrophilic skin layers together with the copenetrating solvent PG. On the one hand, the latter determines the partition behaviour in the way that higher PG diffusion increases penetrant solubility in the skin and lowers the partition coefficient $P_{\text{PBA Stratum corneum/viable skin}}$. On the other hand, it allows cotransport or solvent drag in a narrower sense.

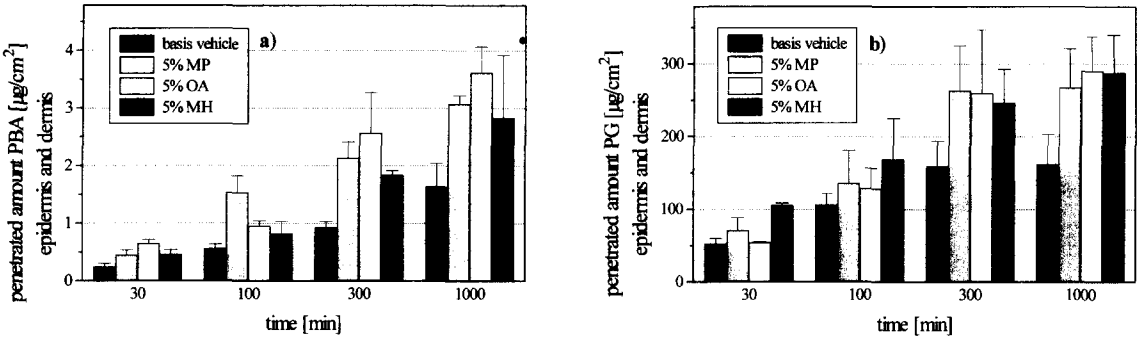

Fig. 5. (a) Influence of the fatty acids on the penetration of PBA into the viable skin, (Each point represents the mean ± S.D., $n = 3$). (b) Influence of the fatty acids on the penetration of PG into the viable skin, (Each point represents the mean ± S.D., $n = 3$).

3.4. Penetration of PG

3.4.1. Stratum corneum

The diagram of the penetrated amount of PG as a function of time shows an optimum curve with its maximum at about 100 min (Fig. 3b). This result must be explained by depletion of the penetrant on the donor side. Caused by PG influx, the amount increases with time and reaches a maximum before it decreases due to an uptake into the viable epidermis. This result is a typical one for a transit compartment. However, it clearly demonstrates that PG easily penetrates into and permeates through human skin (Mollgaard and Hoelgaard, 1983). Evaporation of PG possibly contributes also to the extremely reduced amounts at 1000 min.

There is sufficient indication of enhanced PG partition into the skin when the fatty acids are added. A lag time to the occurance of significant improved levels of PG is in accordance with the theory that fatty acids take some time to enter the skin and to develop their effects ('pull'-effect; Kadir et al., 1988). Furthermore, a 'push'-effect (Kadir et al., 1987), i.e. changes of the thermodynamic condition of PG due to the penetration of the fatty acids themselves has to take into consideration. No differences between the individual compounds are detectable. Therefore, a nonspecific mode of enhancement for PG penetration has to be assumed.

3.4.2. Viable skin

As apparent from Fig. 5b, the enhanced transport of PG into the hydrophilic skin layers continued in the presence of the fatty acids. Earlier studies (Aungst et al., 1990; Nomura et al., 1990) have already reported on the ability of enhancers like fatty acids to improve the penetration of PG. It was also found that PG in turn can promote the modulator penetration (Mahjour et al., 1989). Thus, mutual influences result in continuous changes of the thermodynamic activity within the donor correlated with changes of the partition behaviour of all vehicle compounds as well as in changes of the interaction with the barrier structures.

In our investigation, the same extent of effects is obvious for the branched fatty acids and OA within the viable skin.

Beside the actual experimental data, results with use of fatty acids have much higher standard deviations than the reference. This confirms the high variability of human skin to penetration modulator treatment.

4. Conclusion

According to the literature, it is extensive evidence about the effective enhancement synergism of OA or other fatty acids and PG for lipophilic and moderate polar drugs such as salicylic acid

(Cooper, 1984), betamethasone-17-benzoate (Bennett et al., 1985), nitroglycerin (Loftsson et al., 1987), tetrahydrocannabinol (Touitou and Fabin, 1988) and 17β-estradiol (Loftsson et al., 1989).

From this study it could be concluded that there does not exist any difference in the effectiveness of dermal drug transport using the branched fatty acids or OA as penetration modulators. However, taking into consideration that PBA is a highly lipophilic model substance, the diffusion out of the horny layer is the rate-limiting step for penetration. Therefore, enhancement effects of the fatty acids due to their ability to penetrate the target region, the stratum corneum, and to decrease reversibly the native diffusional barrier for facilitated drug passage might be masked.

The action of the fatty acids appear restricted to the influence on PG penetration under the described experimental conditions. But its improvement correlates directly with an enhancement in PBA penetration refering to a cotransport or solvent drag mechanism. Thus, it has to be concluded that the penetration of very lipophilic substances is rather enhanced nonspecifically. Comparing these results with permeation studies, a detailed evaluation of the participation of both skin compartments differing in characteristics as diffusion medium for penetrants is possible.

Perspectively, the influence of the branched fatty acids and PG on the penetration of a medium lipophilic substance as well as a hydrophilic penetrant has to be investigated. Furthermore, studies on the penetration of the fatty acids themselves into the skin layers are necessary to assess both their penetration modulating properties and interactions with the stratum corneum lipids. In the case of MH, an investigation into the participation of both individual isomers on the total effect of drug penetration enhancement is of interest to elucidate the importance of the kinked *cis* alkenyl chain structure.

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