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Effect of lipophilic vehicles on in vivo skin penetration of methyl nicotinate in different races

Claudia S. Leopold*, Howard I. Maibach

Department of Dermatology, School of Medicine, University of California San Francisco, San Francisco, USA

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Abstract

Studies on racial differences in percutaneous penetration have mainly focused on measurements of either the pharmacodynamic response of a drug or skin reactivity to irritants. In order to clarify the contribution of the barrier stratum corneum to the overall drug penetration rate in different races, an in vivo permeability study was conducted with methyl nicotinate as the model drug. Four races (Caucasians, Hispanics, Blacks, Asians) were investigated. The drug was dissolved in various lipophilic vehicles (dimethicone 100, light mineral oil, isopropyl myristate, medium chain triglycerides) at concentrations that provide equal drug escaping tendencies. Drug solutions were applied to the upper arms of 48 subjects (four races with 12 subjects each) with a glass chamber system. To avoid drug depletion, drug disappearance rates were measured under steady-state conditions by the so-called difference method. Drug flux among the four races increases in the following order: Blacks < Asians < Caucasians < Hispanics. The effect of race almost reaches significance level ($P = 0.067$). Fisher's PLSD post-hoc procedure indicated a significant difference between drug penetration in Hispanics and Blacks ($P = 0.009$) suggesting structural or functional differences in the stratum corneum. Enhancement factors were calculated from the steady-state flux values, (i.e. drug disappearance rates per area unit) for each race. Significant enhancing effects ($P = 0.0001$) were observed with the vehicles isopropyl myristate and mineral oil with all races.

Keywords: Race; Percutaneous penetration; Penetration enhancers; Methyl nicotinate; Glass chamber

1. Introduction

Racial differences in percutaneous penetration of chemicals and drugs have been documented (Stoughton, 1969; Weigand and Gaylor, 1974; Wedig and Maibach, 1981; Guy et al., 1985; Gean et al., 1989; Berardesca and Maibach, 1990).

* Corresponding author. Present address: Department of Pharmaceutical Technology, Heinrich-Heine-University, Universitätsstr. 1, 40225 Düsseldorf, Germany. Tel/fax: +49 211 8114225; e-mail: leopold@uni-duesseldorf.de.

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However, attention has mainly focused on defining different skin permeability in whites and blacks. Investigations with diflorasone acetate revealed no racial differences in penetration between black and white subjects which might have been due to the small number of subjects and the high interindividual variability (Wickema-Sinha et al., 1978). White skin is supposed to be more permeable to certain chemicals than black skin (Stoughton, 1969; Weigand and Gaylor, 1974; Wedig and Maibach, 1981). Racial differences in percutaneous penetration of nicotinates with slightly decreased levels in black skin were found using laser Doppler velocimetry for the assessment of vasodilation (Guy et al., 1985; Gean et al., 1989; Berardesca and Maibach, 1990). Dipyrithione penetration was found to be less in black skin (Wedig and Maibach, 1981). In vitro, the absorption of fluocinolone acetonide was higher in white skin (Stoughton, 1969).

With regard to the barrier stratum corneum it was found that the stratum corneum layer counts were significantly higher among blacks (Weigand et al., 1974). However, the average stratum corneum thickness was similar in blacks and whites, indicating that the black stratum corneum is more compact. Besides this increased intercellular cohesion a higher lipid content in black stratum corneum was observed (Rienertson and Wheatley, 1959). No anatomical and biochemical studies have been published for Hispanic skin.

Racial differences in percutaneous penetration may not only be caused by structural differences in the stratum corneum. Depending on the method used for the determination of drug penetration a difference in persistence or substantivity of the compound, qualitative and quantitative differences in metabolism (Wedig and Maibach, 1981) or, in the case of hyperemic reactions, a different reactivity of the blood vessels (Berardesca and Maibach, 1989) may lead to an over- or underestimation of the actual drug penetration rate.

This study was done to detect differences in percutaneous penetration of methyl nicotinate in four different racial groups (Caucasians, Hispanics, Blacks, Asians) by determining the actual drug flux through the barrier stratum corneum under steady-state conditions. In addition, the effect of several different lipophilic excipients widely used in creams and ointments on percutaneous penetration was investigated.

2. Materials and methods

2.1. Vehicles and model drug

The following lipophilic liquids, arranged in ascending order according to their ability to dissolve the model drug MN, were used: dimethicone 100 (DIM; Baysilone M 100, Bayer AG, Leverkusen, Germany), selected as standard because it was expected not to exert specific vehicle effects due to its high molecular weight of ~ 6700 Da; highly purified light mineral oil (MO; Parafluid Mineralölgesellschaft, Hamburg, Germany); isopropyl myristate (IPM; Henkel KGaA, Düsseldorf, Germany); and caprylic/capric acid triglycerides (CCT; Hüls Troisdorf AG, Troisdorf, Germany). MN was used as model drug because it penetrates rapidly and thus facilitates the measurement of drug fluxes.

2.2. Subjects

Forty-eight healthy female subjects (12 Caucasians, 12 Hispanics, 12 Blacks, and 12 Asians), aged 20–60 years, participated. The thickness of female skin is supposed to be constant within this age range (Shuster et al., 1975). The purity of ethnicity of the groups was such that all four grandparents of each subject demonstrated identical racial characteristics. All volunteers provided written informed consent to participate. They had free access to food and soft drinks during the experiment

2.3. Method of application

Penetration measurements were done with the glass chamber system described previously (Leopold and Lippold, 1992). This method allows the measurement of even small drug fluxes by the so-called difference method (Stricker et al., 1987). Four drug solutions were applied to the upper arms of the subjects and examined under occlusion conditions over 6 h. After a 1-h pretreatment

Table 1
MN solubilities, partition coefficients (vehicle/aqueous phase), relative effective activity coefficients (DIM = standard), and in vivo concentrations for the investigated vehicles

Vehicle	MN solubility (g/ml)	$PC_{V/W}$ 32°C	$\gamma_{T/ST}$	MN conc. applied in vivo (mg/100 ml)
DIM	0.04	0.886	1	32.0
MO	0.07	1.171	0.757	42.3
IPM	0.40	4.710	0.188	170.1
CCT	0.48	7.447	0.119	269.0

period with the respective vehicle, each glass chamber was filled with one of the drug solutions, emptied after 1 h, and refilled with the initial drug solution. Zero-order kinetics were assumed because the concentration decrease in each 1-h time interval was $< 10\%$. The concentration of the donor phase samples was measured spectrophotometrically (Spectronic 1001, Bausch and Lomb, Rochester, NY). MN disappearance rates per area unit were calculated from the concentration differences between the initial solution and the samples obtained after every hour, multiplied by the volume of the respective chambers, and divided by the application area and the time interval. A steady state, indicated by constant drug disappearance rates, was reached after $\sim 1.5-3$ h with all subjects. With hairless rat skin however, it was found that a steady state can be reached only after 4 h (Lafforgue et al., 1995). For further data analysis only the 6-h values were used.

Steady-state drug disappearance rates per area unit are referred to as 'steady-state flux (ss flux)' throughout this paper.

2.4. Penetration measurements and data treatment

Each volunteer received the standard DIM (ST) and the three other vehicles (T). To reach the same thermodynamic activity of the drug, MN concentrations in the vehicles were adjusted according to the reciprocal of the relative effective activity coefficients ($\gamma_{T/ST}$) which equal the ratio of the vehicle/aqueous phase partition coefficients of the standard vehicle ($PC_{ST/W}$) and the test vehicles ($PC_{T/W}$) (Lippold and Reimann, 1989; Leopold and Lippold, 1995b). The amounts of MN that had to be dissolved in the different vehicles were obtained by dividing the initial drug concentration of the standard vehicle, which was 32 mg/100 ml, by the $\gamma_{T/ST}$ values of the test vehicles. Table 1 summarizes these data. Specific vehicle effects, i.e. penetration enhancing effects, manifest themselves in elevated penetration rates and may be quantified by the calculation of enhancement factors EF (Kadir et al., 1988; Aungst, 1989; Ghanem et al., 1992; Leopold and Lippold, 1995b). The latter can be obtained by dividing the steady-state flux values of the test preparations by the values of the standard preparation with the same thermodynamic activity (ss $flux_a$).

2.5. Statistical analysis

Only the last flux values obtained after 6 h were used for statistical evaluation after logarithmic transformation. A 4 × 4 ANOVA was computed with race as grouping factor and vehicle as a repeated measurement factor. Post-hoc comparisons among the four groups utilized Fisher's Protected Least Significant Difference. Statistical analyses were performed using StatView 4.5 and SuperAnova 1.1 for Macintosh. A log normal distribution of the data was assumed in accordance with previously conducted studies (Leopold and Lippold, 1992, 1995b). Variances were considered to be homogeneous on the basis of the results of the Bartlett test.

3. Results and discussion

Most of the published human in vivo percutaneous penetration data is on white skin. The discovery of major racial differences in percuta-

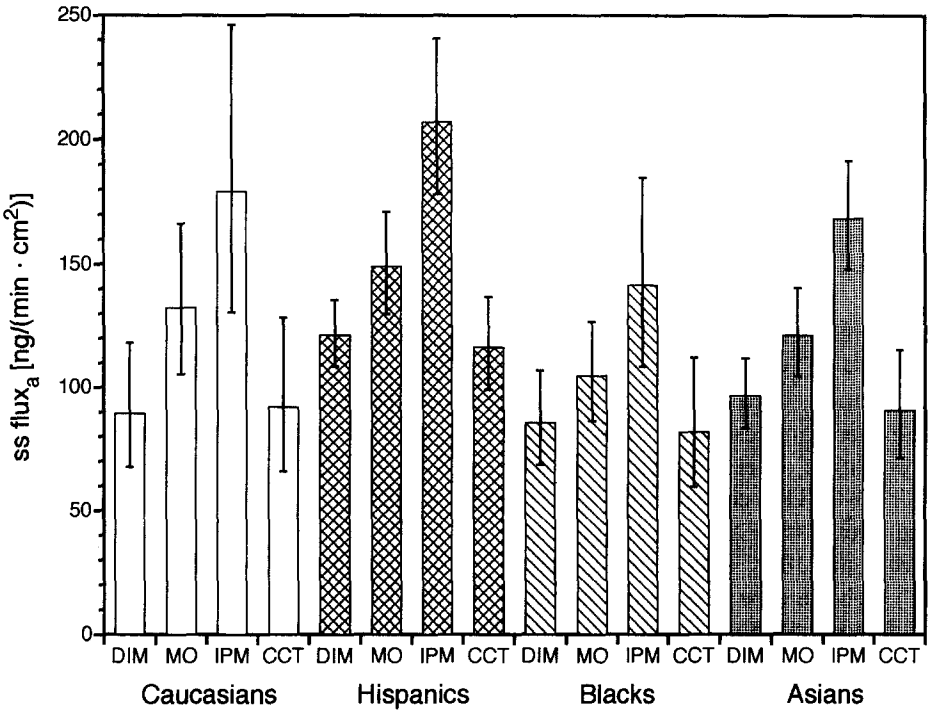

Fig. 1. Steady-state-flux values (ss flux$_a$) for four different races from different vehicles with the same drug activity. DIM = standard, $\bar{x} \pm$ 95% confidence limits, $n = 4 \times 12$ subjects.

neous penetration would undoubtedly lead to consequences such as an adjustment of the drug dose or an alteration of the ointment base in topically applied ointment preparations for each race. Few studies have been conducted to investigate racial differences in skin penetration (Wedig and Maibach, 1981; Guy et al., 1985; Berardesca and Maibach, 1988; Berardesca and Maibach, 1989; Gean et al., 1989; Berardesca and Maibach, 1990). Many of them did not result in statistically significant differences which might be due to the small number of subjects and the high interindividual variability.

The overall goal of this study was to ascertain whether the permeability characteristics of Hispanic, black and Asian skin are similar to those of white skin. In order to get an objective measure of the amount of drug actually penetrated, the flux of a model drug through the skin was measured over a time period of 6 h as drug disappearance rates. The resulting flux values should allow detection of possible racial differences in the barrier properties of the stratum corneum.

The results of the penetration experiments are shown in Fig. 1. It appears that the drug flux increases in the following order: Blacks < Asians < Caucasians < Hispanics. The main effect for race almost reaches significance level (F[3,44] = 2.56; P = 0.067). However, Fisher's Protected Least Significant Difference procedure indicated a significant difference between drug penetration in Hispanics and Blacks (P = 0.009). There was no interaction between race and vehicle (F[9,132] = 0.85; P = 0.57).

The data presented here are consistent with literature data. It is known that black skin is less permeable to various compounds than white skin (Stoughton, 1969; Weigand and Gaylor, 1974; Wedig and Maibach, 1981). Similar results were found with nicotinates in pharmacodynamic studies, although less pronounced (Guy et al., 1985; Gean et al., 1989; Berardesca and Maibach, 1990). This decreased permeability of black skin may be due to the more compact structure of the

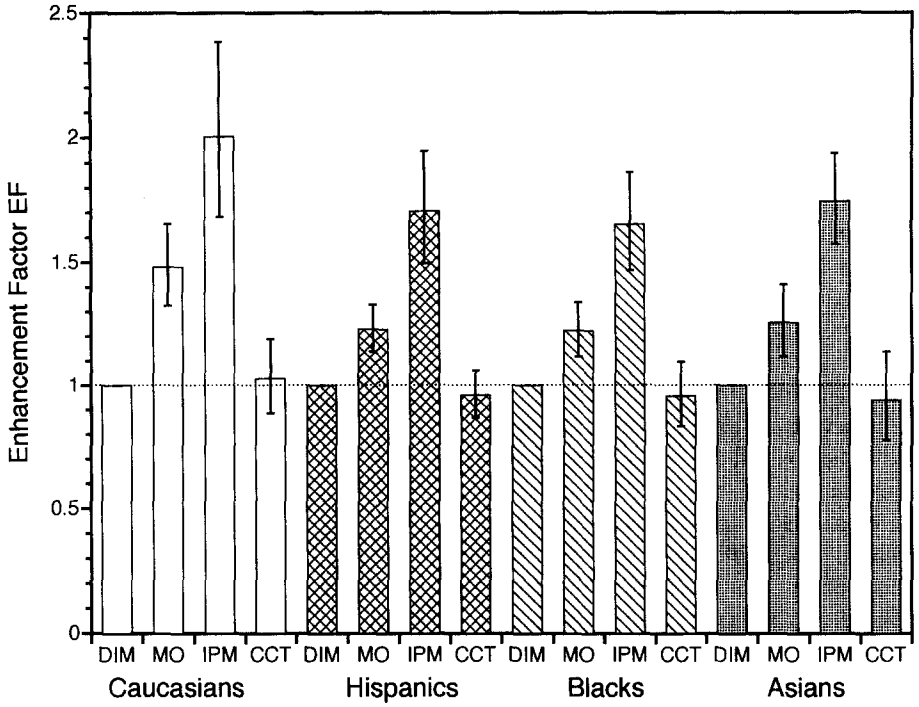

Fig. 2. Enhancement factors EF calculated from the data shown in Fig. 1 (EF = ss $flux_{a\ T}$/ss $flux_{a\ ST}$); 95% confidence limits, $n = 4 \times 12$ subjects.

black stratum corneum (Weigand et al., 1974) or a higher lipid content of the horny layer of blacks (Rienertson and Wheatley, 1959).

The high flux values found with Hispanic skin are somewhat surprising (Fig. 1). No difference in percutaneous penetration of nicotinates was found between whites and Hispano–Americans using laser Doppler velocimetry to assess vasodilatation (Berardesca and Maibach, 1988). Moreover, skin thickness and TEWL values are similar in white and Hispanic skin (Berardesca et al., 1991). This apparent contradiction may be due to the different methods used for the measurement of drug penetration. One advantage of the measurement of the drug flux is that possible differences in blood vessel reactivity to nicotinates do not impact the results. However, the differences found here between whites and Hispanics did not reach statistical significance.

As expected, with Asian skin, higher drug penetration rates than with black skin (nonsignificant) and slightly lower penetration rates than with Caucasian skin were observed (Fig. 1). However, with laser Doppler velocimetry it was found that the area under the LDV response versus time curve was greater in Asians than in Caucasians (Gean et al., 1989). Again note that data obtained by laser Doppler velocimetry may not only reflect drug penetration through the barrier stratum corneum but also detects differences in microvasculature sensitivity to nicotinates.

With planned comparisons, significant vehicle effects ($P < 0.0001$) could be found for MO and IPM in comparison to DIM (Fig. 1), indicating that the steady-state penetration rates of MN from these vehicles were higher than expected from the thermodynamic activities of MN in the latter. This is true for all races as no interaction was observed between race and vehicle. The calculation of enhancement factors allows a quantification of specific vehicle effects (Fig. 2). In all four races a similar effect on the stratum corneum structure can be observed with MO and IPM, respectively. With Caucasian skin, slightly greater

EF values were found with MO and IPM (Fig. 2) compared to the other races, indicating a higher susceptibility of white skin to barrier disruption by these two penetration enhancers. However, the enhancing effects of both vehicles are not pronounced, with EF values of 1.2–1.5 and 1.7–2.0 for MO and IPM, respectively. They may be explained by an interaction of the vehicles with the stratum corneum lipids (Leopold and Lippold, 1995b). This interaction leads to a decrease of the stratum corneum resistance as shown by differential scanning calorimetry measurements in white skin (Leopold and Lippold, 1995a). The alteration of the lamellar structure of the stratum corneum lipids may occur either by a fluidizing action or by dissolution or extraction of the lipids (Leopold and Lippold, 1995a). Both processes ultimately lead to an increased drug diffusivity and/or drug solubility in the barrier. In summary, the presented data indicate that there may be differences in percutaneous penetration between different races. A statistically significant difference could be found between Hispanic and Black subjects which may be explained by structural or functional differences in the barrier stratum corneum. Increasing the subject population would probably lead to significant differences between all four races. It could be shown that mineral oil and isopropyl myristate act as penetration enhancers for methyl nicotinate not only in Caucasians but also in Hispanics, Blacks and Asians. However, additional compounds must be studied to ascertain if the differences noted here are compound specific or a general phenomenon.

Acknowledgements

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