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Influence of physicochemical properties of homologous esters of nicotinic acid on skin permeability and maximum flux

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Abstract

The uptake of homologous esters of nicotinic acid by the skin was investigated with glass chambers on 15 healthy volunteers. The permeabilities P_B and maximum fluxes J_{max} were calculated from the concentration decrease of the aqueous solutions after fixed periods of time. A linear relationship was established between log P_B and log $PC_{Oct/W}$, the octanol/water partition coefficient. The slope of 0.32 of the plot log P/log $PC_{Oct/W}$ was lower than the theoretical value of 1 in the case of membrane control assuming a liquid octanol membrane. This large deviation is a consequence of a distinct difference between the lipophilicity of the lipid regions of the stratum corneum and octanol. Therefore, no clear dependence was observed between the maximum flux J_{max} and the octanol solubility c_{sOct} of the esters. However, a linear relationship resulted in the plot of log J_{max} + (1 − 0.32) log $PC_{Oct/W}$ vs log c_{sOct}, taking into account the relation between P_B and $PC_{Oct/W}$. Thus, the maximum flux of a drug may be predicted knowing its physicochemical properties.

Keywords: Nicotinic acid, homologous ester; Lipophilicity; Maximum flux; Partition coefficient; Skin permeability; Solubility

1. Introduction

There have been several recent reports on structure-penetration relationships (Flynn, 1990; Dal Pozzo et al., 1991b; Tsai et al., 1992; Hadgraft and Wolff, 1993; Potts and Guy, 1993; Surber et al., 1993; Weber et al., 1994). The basis of those studies is to establish a correlation between the skin permeability (P_B) and physicochemical properties, such as the octanol/water partition coefficient ($PC_{Oct/W}$). The results are useful for predicting drug transport across membranes. Moreover, information about the mechanism of passive diffusion and the role of aqueous boundary layers and membrane control can be gained (Díez-Sales et al., 1991).

Transdermal drug transport can be described following Fick's first law, assuming the stratum corneum to be a homogeneous lipid membrane (Poulsen, 1972; Kasting et al., 1987). Thereby, linear correlations between the logarithms of human skin permeability P_B of many compounds and the logarithms of $PC_{Oct/W}$ can be observed (Roberts et al., 1978; Bronaugh and Congdon, 1984; Anderson et al., 1988). Only extremely hy-

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drophilic and lipophilic species may show deviations: Hydrophilic molecules possibly penetrate through pores. The transport of highly lipophilic molecules may be hindered by aqueous boundary layers. Hence, sigmoidal or hyperbolic dependencies have been observed (Scheuplein, 1965; Roberts, 1985; Anderson et al., 1988; Del Terzo et al., 1989; Flynn, 1990; Dal Pozzo et al., 1991a). Moreover, a parabolic or bilinear relationship between P_B and $PC_{Oct/W}$ is postulated and has been detected, the latter predominantly with pure drugs or suspensions, using flux values instead of P_B (Hansch, 1969; Flynn and Yalkowsky, 1972; Stehle and Higuchi, 1972; Kubinyi, 1979; Yano et al., 1986; Houk and Guy, 1988; Dal Pozzo et al., 1991a; Díez-Sales et al., 1991; Surber et al., 1993).

In practice, however, the concept of maximum flux is of most interest when attempting the uptake of drugs through the skin (Lippold, 1992; Cooper, 1986). Since these molecules are often similar in size, it is reasonable to assume the maximum flux (from a saturated solution) to be a function of the drug solubility in the stratum corneum (Scheuplein, 1965), simply expressed as octanol solubility (Kasting et al., 1987).

Therefore, the aim of this investigation was to determine the influence of physicochemical properties of homologous esters of nicotinic acid as model drugs on skin permeability. Furthermore, it was of interest to study whether a simple relationship exists between octanol solubility and maximum flux. A successful correlation could possibly enable the prediction of maximum drug uptake from knowledge of the physicochemical parameters.

The volunteers consisted of Europeans and Asians. The aim was to investigate whether differences were detectable in the penetration behaviour of both groups.

2. Materials and methods

2.1. Homologous esters of nicotinic acid

Methyl (MN), butyl (BN), hexyl (HN) and octyl nicotinate (ON) (Aldrich-Chemie, Germany). Table 1 shows the relevant physicochemical data of the esters.

Table 1
Physicochemical properties of the homologous esters of nicotinic acid

Ester	MW	$PC_{Oct/W}$	c_{sW} (mg ml^{-1})	c_{sOct} (mg ml^{-1}]
MN	137	7	1106	7624
BN	179	292	2.45	715
HN	207	3233	0.17	548
ON	235	51182	0.01	527

MW, molecular weight; $PC_{Oct/W}$, octanol/water partition coefficient at 32°C; c_{sW}, and c_{sOct}, solubility in water and octanol, respectively, at 32°C.

The esters were applied in an aqueous solution, phosphate buffer pH 5.5 DAB 10. The initial concentration was 5×10^{-4} mol l^{-1} for MN, BN, HN and 3×10^{-5} mol l^{-1} for ON.

2.2. Application chamber

The penetration study of nicotinic acid esters was carried out with glass chambers with a high area/volume quotient and a good sealing capacity (Leopold and Lippold, 1992). The application area was nearly 14 cm^2, the volume varying between 3 and 5 ml.

2.3. Volunteers

The first group consisted of 10 Europeans (three males, aged between 23 and 40), the second of five Asians (three males, aged between 12 and 40). All of them were healthy and non-smokers. Two chambers were fixed on each upper arm, so that the four esters (MN, BN, HN and ON) could be investigated at the same time.

An earlier study (Leopold, 1992) showed that the position of this chamber was statistically not significant with respect to the penetration rate. Therefore, the chambers were placed randomly on the right or left upper arm at a higher or lower position and fixed with a band.

2.4. Analytics

The decrease in concentration of the esters in the solution was determined by HPLC: Shimadzu LC-6A (Duisburg, Germany), with an automatic

injector (SIL-6 B, SCL-6 B), an integrator and a plotter (CR 4 AX Chromatopac) and UV detector (SPD, 6 AV); volume of injection, 20–100 μl; detection, 263 nm; column, Lichrospher® 100 RP 18, 5 μm, 125 × 4 mm (Merck, Darmstadt, Germany); flow rate, 1.5–2 ml/min; attenuation, 2^5 mV; mobile phase, methanol/water 1:1 (MN, BN), 3:1 (HN), 4:1 (ON); standard, ethyl nicotinate as internal standard for MN and BN, external standard for HN and ON (Le, 1993).

2.5. Penetration kinetics and calculation of skin permeability

According to preliminary experiments (Le, 1993), the concentration decrease in the chambers during an interval of 1 h proceeds exponentially so that first-order kinetics can be assumed. In vivo studies with a solution of methyl nicotinate on a guinea-pig have also indicated that the uptake takes place according to first-order kinetics (Osamura et al., 1984). In the course of this study the chambers were emptied every hour and refilled five times with the initial solution. The concentration decrease per h was practically constant, so that a mean value could be calculated over the period of 5 h. This mean decrease in concentration of each volunteer, multiplied with the actual volume V of the chamber and divided by the area of application A gave the mean disappearance rate of the drug or flux J of each particular volunteer. Their average over all volunteers was the mean flux J for 1 h of the different esters. However, the resulting fluxes for 1 h comprised exponential decreases in concentration and were valid for the applied concentration only. For the purpose of better comparability, the penetration rate constant k_p of each volunteer was calculated after every interval of 1 h (Eq. 1 and 2) and averaged for the five intervals. These values were still dependent on the size of each chamber. Thus, the k_p values were multiplied with the volume V and divided by the application area A of the chamber used to yield the mean permeability for each volunteer and each ester (Eq. 3):

$$k_p = (\ln c_0 - \ln c_t)/t \quad (1)$$

for $t = 1$ h

$$k_p = \ln c_0 - \ln c_t \text{ with } k_p \text{ as } [h^{-1}] \quad (2)$$

$$P_B = \frac{k_p \cdot V}{A} \quad (3)$$

where c_0 and c_t denote the initial concentration and the concentration at time t, respectively.

3. Results and discussion

3.1. Fluxes and permeabilities of homologous esters of nicotinic acid

Table 2 lists the mean values of the disappearance rate or fluxes, respectively, with the standard deviation on a mass and molar basis for Europeans and Asians. All in all, the fluxes were very high. For example, the flux obtained from a saturated solution of scopolamine, which is generally regarded as a good penetrant, was only 0.0125 μmol cm^{-2} h^{-1} (Michaels et al., 1975).

Fig. 1 shows the permeabilities of the Europeans and Asians. Similar to the flux values, the permeabilities P_B increased clearly with increas-

Table 2
Mass and molar fluxes (J_{mass}, J_{molar}) and permeabilities P_B in vivo of homologous esters of nicotinic acid (mean ± S.D.) and calculated maximum mass fluxes J_{max}; $n = 15$

Ester	J_{mass} (μg cm^{-2} h^{-1})	J_{molar} (μmol cm^{-2} h^{-1})	P_B (10^{-2} cm h^{-1})	J_{max} (μg cm^{-2} h^{-1})
MN	1.35 ± 0.55	0.010 ± 0.004	0.89 ± 0.514	9799.2
BN	6.31 ± 1.88	0.035 ± 0.014	3.34 ± 1.40	81.9
HN	12.87 ± 2.83	0.062 ± 0.021	8.14 ± 2.49	13.85
ON	22.67 ± 5.45 [a]	0.096 ± 0.023 [a]	14.81 ± 2.18	1.48

[a] Extrapolated to the same initial concentration as the other esters.

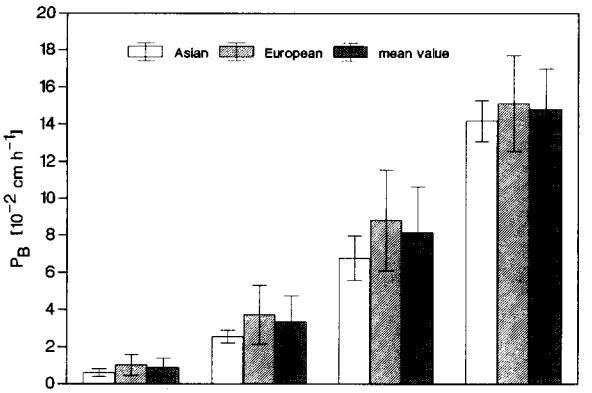

Fig. 1. Comparison of the permeability of Asians ($n = 5$) and Europeans ($n = 10$) and mean value of the permeability P_B of all 15 volunteers with standard deviation.

ing alkyl chain length, from methyl nicotinate to octyl nicotinate by a factor of 15. The permeabilities of the Europeans appeared to be a little greater than for the Asians. However, this effect was not significant (t-test, $P \leq 0.05$), which is in accordance with results from penetration studies of corticosteroids (Wester and Maibach, 1991). For the following correlations and discussions, the mean values of permeabilities of all volunteers were referred to (Fig. 1 and Table 1). The P_B values of MN, BN and HN were of the same order of magnitude, but about 70% greater than those from in vitro studies (Dal Pozzo et al., 1991a)

3.2. Correlation between permeability P_B and lipophilicity

The permeabilities of the drug include its diffusion coefficient in the barrier stratum corneum D, its partition coefficient between the stratum corneum and the aqueous vehicle $PC_{Str.\ corn./W}$ and the thickness of the barrier l:

$$P_B = \frac{D \cdot PC_{Str.\ corn./W}}{l} \quad (4)$$

Provided the permeabilities obtained are controlled by aqueous boundary layers, the diffusion coefficients in water and the diffusion path length in the aqueous layers determine P_B, and $PC_{Str.\ corn./w}$ is without influence.

As shown in Fig. 2, the skin permeabilities increase linearly with extension of the alkyl chain length (increasing lipophilicity) in this semilogarithmic plot. However, only the first three esters (MN, BN and HN) lie exactly on a straight line, the octyl homologue deviating a little. All together, the permeabilities have values within only one order of magnitude.

The linear relationship between the logarithm of the permeabilities and the number of carbon atoms in the alkyl chain for MN, BN, HN and ON is the result of a thermodynamic group contribution to the partitioning with prolongation of the chain length (Davis et al., 1972). The slope of the plot in Fig. 2 gives the incremental Hansch constant π, which has a value of only 0.18.

Assuming that the stratum corneum may be regarded as a lipophilic partitioning membrane, whose lipids behave like octanol, the slope of the semilogarithmic plot in Fig. 2 should be the same as the π constant 0.55 of the octanol/water partition system (Le, 1993). The small slope found in vivo for the nicotinic acid esters could be due to their low affinity to the stratum corneum in comparison with octanol (Roberts, 1991). In penetration studies with alkanols on excised human skin (Scheuplein, 1965) and hairless mouse skin (Durrheim et al., 1980), π values of 0.33 and 0.26

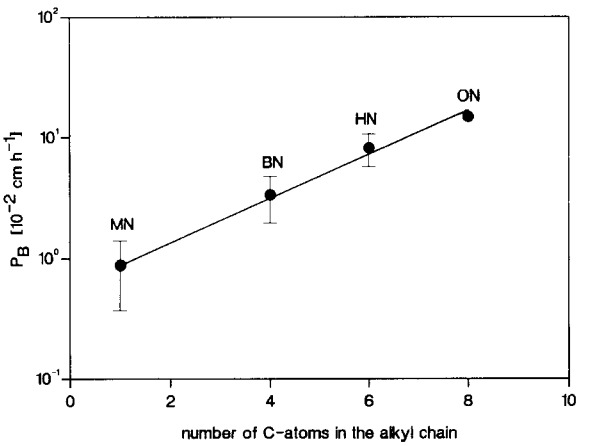

Fig. 2. Relationship between the logarithm of the permeability and the alkyl chain length; mean value with standard deviation, $n = 15$.

were found. Generally, a π value between 0.2 and 0.3 is proposed for biological membranes of the skin and mucosa type (Flynn and Yalkowsky, 1972, Yalkowsky and Flynn, 1973; Flynn et al., 1974).

For the discussion of the permeability/lipophilicity relationship for substances not descending from a homologous series, the octanol/water partition coefficient is used as a measure of lipophilicity. Assuming a similar size of the diffusing molecules, Eq. 4 may be written as:

$$P_B = \text{const.} \cdot PC_{\text{Str. corn./W}} \quad (5)$$

and

$$\log P_B = \log \text{const.} + \log PC_{\text{Str. corn./W}} \quad (6)$$

Eq. 6 is converted by means of the Collander equation (Collander, 1947, 1950) to:

$$\log P_B = \log \text{const.} + b + a \log PC_{\text{Oct/W}}$$
$$= \text{const.}' + a \log PC_{\text{Oct/W}} \quad (7)$$

The constant a in Eq. 7 describes the selectivity of the stratum corneum, which means its sensitivity to a change in the lipophilicity of the solutes, in comparison to octanol for the investigated substances (Leo and Hansch, 1971; Diamond and Katz, 1974). Small a values ($a < 1$) indicate a lower affinity of the drugs to octanol than to the stratum corneum. In the case of nicotinic acid esters an a value of 0.32 is obtained, implying lower lipophilicity of the stratum corneum with regard to octanol. The deviation of the points from the straight line does not change significantly on consideration of a different molar volume V_M, the slope rising to 0.35 (Le, 1993). In corresponding literature, a values from 0.48 to 1.2 can be found. However, these data were obtained from experiments with excised human skin (Roberts et al., 1978; Bronaugh and Congdon, 1984; Roberts, 1985, Anderson et al., 1988). Recently, Potts and Guy (1993) postulated a value of 0.71 for human skin in vitro.

In same cases (phenols, alkanols and steroids) earlier penetration studies on excised skin have shown that a plateau of the $\log P_B/\log PC_{\text{Oct/W}}$ profile is obtained with higher members of the homologous series (Scheuplein, 1965; Roberts et al., 1985; Anderson et al., 1988; Del Terzo et al., 1989; Flynn, 1990; Dal Pozzo et al., 1991b). A parabolic or bilinear relationship between the logarithms of the permeabilities and those of the octanol/water partition coefficients, with the substance applied as a diluted solution, has not yet been clearly established (Houk and Guy, 1988, Flynn, 1990). On the other hand, a parabolic dependence between the log permeability and $\log PC_{\text{Oct/W}}$ resulted from penetration studies with homologous esters of nicotinic acid, applied as pure liquid on excised skin (Dal Pozzo et al., 1991b). Aqueous boundary layers are assumed to exist in the viable epidermis (Houk and Guy, 1988; Dal Pozzo et al., 1991a; Díez-Sales et al., 1991), which could be a possible reason for the optimal permeability of compounds with intermediate $PC_{\text{Oct/W}}$ values or for solubility-limited transport (Hansch, 1969; Flynn and Yalkowsky, 1972; Stehle and Higuchi, 1972; Kubinyi, 1979). However, as a result of vasodilatory effects, the esters of nicotinic acid could influence the cutaneous blood supply favourably in vivo, so that rapid removal of the drug should be considered for these compounds. As a consequence, a deviation from linearity can possibly be shifted to higher members of the homologous series. A study with completely different substances in propylene glycol/water mixtures again revealed a straight line with a slope of 0.38 in the $\log P/\log PC_{\text{Str. corn./Vehicle}}$ plot, using the same in vivo method (Hagedorn-Leweke and Lippold, 1995).

3.3. Maximum flux

The maximum disappearance rate or flux J_{max} (flux/cm^2 per h from a saturated solution) was calculated based on the respective permeability P_B and solubility c_{SW} ($J_{max} = P_B \cdot c_{SW}$). Table 2 lists the data on a mass and molar basis, respectively, with standard deviations. The maximum flux decreases in the order MN, BN, HN, ON. Methyl nicotinate has the highest maximum flux, the smallest $PC_{\text{Oct/W}}$ and P_B, but the highest water and octanol solubility.

These calculated J_{max} data correspond well with the results from an in vitro study with ex-

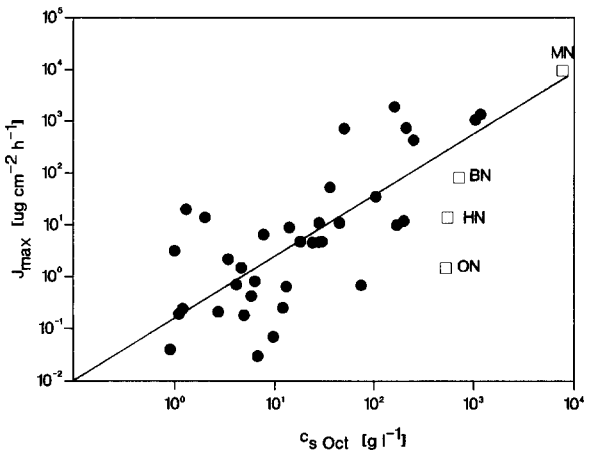

Fig. 3. Correlation between maximum flux and octanol solubility of nicotinic acid esters, plotted together with the in vitro data from Kasting et al. (1987); a straight line with a slope of unity is plotted in the graph, assuming membrane control and the same properties of stratum corneum lipids and octanol.

cised skin (Dal Pozzo et al., 1991b). For example, butyl nicotinate has a maximum flux of 79 μg cm^{-2} h^{-1} in vitro and 82 μg cm^{-2} h^{-1}, calculated from the in vivo permeability, and hexyl nicotinate 12.2 μg cm^{-2} h^{-1} in vitro and 13.8 μg cm^{-2} h^{-1} calculated. This indicates that the calculation method provides reliable values.

In order to test the hypothesis of Kasting et al. (1987) of the direct interdependence of J_{max} and c_{sOct}, the obtained maximum fluxes of the nicotinic acid esters were plotted vs their octanol solubility on a log-log scale, together with the in vitro data of Kasting et al. (Fig. 3). It is evident that the maximum fluxes generally increase with increasing octanol solubilities. However, a linear dependence between the logarithms of both parameters is not recognizable for the esters of nicotinic acid. A steep increase in J_{max} is observed in the upper solubility range with only a slightly rising octanol solubility, after which the flux of methyl nicotinate levels off. This indicates that the hypothesis of Kasting et al. is only a rough simplification of the existing dependence. It is noticeable that especially the lipophilic homologous members deviate from the postulated straight line with a slope of 1.

The plot according to Fig. 3 does not take into consideration that octanol simulates the stratum corneum only to some extent. Referring to a more rigorous treatment (Hagedorn-Leweke and Lippold, 1995), $J_{max} = P_B \cdot c_{sW}$ may be expressed with the help of Eq. 7 as:

$$\log J_{max} = \text{const.}' + a \log PC_{Oct/W} + \log c_{sW} \quad (8)$$

with $b = 0.32$ for the esters of nicotinic acid. According to $PC_{Oct/W} = c_{sOct}/c_{sW}$, c_{sW} may be expressed as $c_{sW} = c_{sOct}/PC_{Oct/W}$. This leads to the following:

$$\begin{aligned}\log J_{max} &= \text{const.}' + 0.32 \log PC_{Oct/W} \\ &\quad - \log PC_{Oct/W} + \log c_{sOct} \\ &= \text{const.}' - 0.68 \log PC_{Oct/W} + \log c_{sOct}\end{aligned} \quad (9)$$

$$\log J_{max} + 0.68 \log PC_{Oct/W} = \text{const.}' + \log c_{sOct} \quad (10)$$

Plotting $\log J_{max} + 0.68 \log PC_{Oct/W}$ vs $\log c_{sOct}$ (Fig. 4), a straight line with a slope of practically 1 and intercept const.' is obtained (Fig. 4). This concept is also valid for other substances in an aqueous solution, for instance, sunscreens (Hagedorn-Leweke and Lippold, 1995). Thus, the maximum flux J_{max} of a drug from a vehicle may be predicted knowing its physicochemical proper-

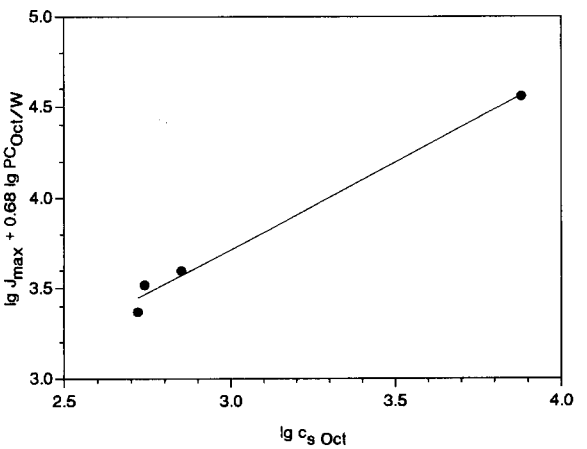

Fig. 4. Correlation between $\log J_{max} + 0.68 \log PC_{Oct/W}$ and $\log c_{sOct}$ according to Eq. 10.

ties, i.e., solubilities and the octanol/water partition coefficient or octanol/vehicle partition coefficient, respectively.

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