J. Pharm. Pharmacol. 1985, 37: 771–775
Received May 3, 1985

Physicochemical characterization of the human nail: solvent effects on the permeation of homologous alcohols*

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To assess how vehicles might influence permeation through human nail, the diffusion of homologous alcohols (methanol to decanol) administered as neat liquids through finger nail plate has been studied using in-vitro diffusion cell methods and compared with permeation data for the same compounds in aqueous media. Permeation rates of the homologous alcohols through lipid depleted nail plate have also been assessed and the influences of dimethylsulphoxide (DMSO) and isopropyl alcohol on permeation rates of methanol and hexanol have been examined. With the exception of methanol, permeability coefficients are uniformly about five-fold smaller when the alcohols are undiluted than when they are applied in water. Overall parallelism in the permeability profiles under these separate circumstances of application is an indication that the external concentrations of the alcohols themselves are a determinant of their permeation velocities through the nail plate matrix. The even separation of the profiles suggests a facilitating role of water within the nail matrix. Chloroform/methanol delipidization of the nail led to increased penetration rates of water, methanol, ethanol and butanol. On the other hand, it caused a six-fold decrease in the permeation rate of decanol. Application of methanol and hexanol in DMSO somewhat retards their rates of permeation. Isopropyl alcohol also slows the permeation rate of hexanol but has little influence on that of methanol. Thus it appears that solvents which tend to promote diffusion through the skin horny layer have little promise as accelerants of nail plate permeability.

Complex influences of DMSO and other organic solvents on the permeability of skin have been demonstrated (Kligman 1965; Dugard & Embery 1969; Scheuplein & Ross 1970; Scheuplein & Blank 1973: Astley & Levine 1976). Depending on the balance of factors, solvents may enhance or retard penetration. In-vivo and in-vitro experiments with DMSO and like solvents indicate they may act to enhance skin permeation by several mechanisms, including extraction of the stratum corneum lipids (Kligman 1965; Dugard & Embery 1969), displacement of stratum corneum bound water (Scheuplein & Ross 1970), the latter possibility being accompanied by keratin denaturation§, and stratum corneum delamination (Chandrasekaran et al 1977). Yet, even with all these factors operating, decreased skin permeation of lipophilic compounds can still occur as the result of exaggeratedly reduced thermodynamic activity of the permeants in their solubilized states in such solvents (Gatmaitan et al 1979). Regardless, it is clear that there are actual permeation altering effects of organic solvents within the horny layer structure which in large measure depend on reorganization of lipid and fibrous protein and displacement of water.

As a closely corresponding anatomical structure human nail might at first be expected to exhibit barrier behaviour paralleling that of the stratum corneum. Nail plate, however, contains far less lipid than found in the skin's horny layer and it also has far less ability to absorb water (Baden et al 1973) and other materials (Kligman 1965) suggesting its protein regime is also molecularly different from that of the stratum corneum. The inherent differences in nail are evident in its unusual structure-permeability sensitivities to the homologous alkanols (Walters et al 1981, 1983). It can be reasoned on these bases that the nail might act differently in the presence of penetration enhancers, but this idea has yet to be tested.

Earlier, the construction of, and technique of use

* This work supported through the generosity of Ortho Pharmaceutical Corporation, Raritan, New Jersey. Presented in part at the British Pharmaceutical Conference, Brighton, UK, 1981.

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§ Recent work in the laboratories of the College of Pharmacy, University of Michigan strongly indicates that denaturation is a major factor influencing the permeation facilitating role of DMSO.

of, a special diffusion chamber for studying nail plate permeability in-vitro was described (Walters et al 1981, 1983). We have now used this cell to characterize solvent influences on the permeation behaviour of the n-alkanols. The alkanols were applied to the nail surface as neat liquids. These data were compared with past results (Walters et al 1983) where the alkanols were applied as highly dilute aqueous solutions. Permeation of select alkanols through chloroform–methanol delipidized nails was then examined, as was the permeability rates of methanol and hexanol from binary aqueous solutions of DMSO and isopropyl alcohol.

MATERIALS AND METHODS

Tritiated water and radiolabelled alcohols were obtained from New England Nuclear ([^{3}H]water, [^{3}H]methanol, [^{14}C]ethanol, [^{14}C]butanol) and ICN ([^{14}C]hexanol, [^{14}C]octanol, [^{14}C]decanol). Reagent grade pure alcohols were used throughout.

Permeation procedures

Details of the diffusion cell and permeation procedures have been given previously (Walters et al 1981). Briefly, trimmed human nail plate sections were placed between two halves of a diffusion cell. A known amount of radiolabelled permeant in solution in either its corresponding neat alcohol or a binary (DMSO/saline, isopropyl alcohol/saline) solvent mixture was placed in the donor chamber and samples were taken at predetermined intervals from the receptor chamber. Isotope activity was monitored using a Beckmann LS 9000 liquid scintillation counter. In all the experiments the receptor chamber was filled with normal saline.

Extraction of nail lipids

Nail plates were immersed in a chloroform–methanol (3 : 1) mixture for 24 h to dissolve and remove accessible lipids. After this extraction the nail plates were rinsed in four changes of saline and placed in the diffusion cells as described above.

Data handling

Permeability coefficients (P) were calculated from:

$$P = V\frac{dC/dt}{A \cdot \Delta C} \quad (1)$$

Where V is the volume of the receiver half cell, dC/dt is the rate of change in concentration in the pseudo-steady state portion of the receiver concentration versus time plot, A is the diffusional area and ΔC is the concentration differential of permeant across the membrane. V(dC/dt) gives the diffusion flux in mass per unit time. The diffusion cells with nail plate membranes in place were scrupulously checked for intercompartmental leakage using soluble but impenetrable polyethyleneglycol markers. No leaks were evident.

RESULTS AND DISCUSSION

It is obvious from Fig. 1 that the unique ability of the nail plate to restrict increasingly the diffusive passage of low molecular weight homologous alcohols as the alkyl chain is lengthened, seen previously when the alkanols were applied as highly dilute aqueous solutions (Walters et al 1983), carries over to the circumstance when the alcohols are applied as neat liquids. Except for methanol, mass transfer coefficients are uniformly about five times smaller when the pure liquids are permeating nail than when the alcohols are in dilute aqueous solution. Since permeability coefficients are concentration normalized mass transfer parameters, the observed differences signify that the actual molecular rates of penetration are five-fold smaller when nail is exposed to the neat alcohols than when the nail is exposed to the alcohols under hydrating conditions. The chemical potential difference between the respective pure liquid states of the alcohols and their respective high aqueous

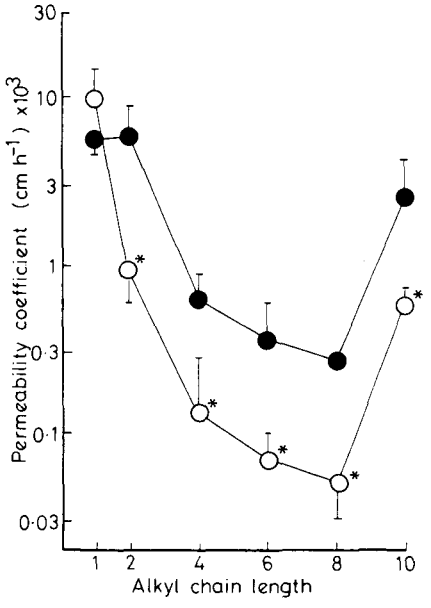

FIG. 1. Permeability coefficients of the n-alkanols through nail plate from dilute aqueous solutions (●) and from neat alcohols (○) as a function of alkyl chain length. Error bars indicate standard deviation. * Indicates significant differences ($P < 0.05$).

dilutions is known to change rapidly as the alkyl chain is lengthened as the alkanols exponentially become less and less soluble in water. On this basis one might expect the separation between the two permeability coefficient curves shown in Fig. 1 to systematically widen with increasing alkyl chain length, but this does not appear to occur. Rather, the roughly five-fold spread in values is maintained past methanol despite exponentially increasing o/w partitioning. A highly 'polar route' through the nail plate capable of excluding permeants on the basis of their relative hydrophobicities was suggested as controlling the permeation of the alcohols, through octanol, when administered in water (Walters et al 1983). This suggestion was made in part because diffusivities of the alkanols in the nail plate matrix calculated from lag times were not greatly different from one another. Incrementally decreasing membrane/water partition coefficients were thus postulated to explain the sharp and systematic permeability coefficient decline seen from methanol to octanol (Fig. 1, upper curve). It is notable that lag times in the present studies, while not documented, were not longer than those seen before. If the separation in the curves in Fig. 1 were strictly brought about by decreased diffusivity as the result of hydration, roughly five-fold longer lag times should have been observed with the neat alcohols. Thus, the permeability behaviour of the neat liquid alkanols to octanol seems to have no simple thermodynamic (partitioning) or kinetic (diffusivity) explanation to tie in neatly with the previous study performed with dilute aqueous solutions. However, the data reported here for the nail plate are to an extent compatible with diverse observations which suggest that the lower alcohols have a diminishing capacity to solvate hard horny tissue as the alkyl chain is lengthened (Harrison & Speakman 1958; Tillman & Higuchi 1961; Wu 1983). In this sense nail plate is more like callus and hair (wool) than it is like stratum corneum. Upon considering chemical compositions of these tissues, the stratum corneum seems to be set apart from the others behaviourally through its high concentration of lipids (Baden et al 1973).

Whether administered in dilute aqueous solution or as the neat alcohol, decanol's behaviour is experimentally set apart from that of the lower alkanols (Walters et al 1983). Though molecularly larger, it proves a better permeant than immediately lower alkanols, because of its higher mass transfer coefficients irrespective of the method of application. Dodecanol behaves similarly whether administered in water (previous study) or in a decanol medium (not reported). The new data do not appear totally compatible with the previously proposed idea (Walters et al 1983) that there is a non-structurally unique parallel lipid pathway which these long chain compounds preferentially, diffusionally negotiate, because neat octanol, for example, would have equal access and facility of diffusion were such a route to exist. It was previously reasoned that exponentially increasing oil/water partition coefficients finally became sufficient to push the higher alcohols across the presumed intercellular lipid route, but partitioning out of the neat alcohols would not follow the same relationship. Thus it appears there is a regime of diffusion offering selective access to the C_{10} and C_{12} alkyl chain length compounds. There remains the impression that some more specific structural requirement is met by decanol and dodecanol which affords them greater solubility or mobility in this phase of the nail.

The nail plate comprises less than 1% lipid (Baden et al 1973) compared with the ≈10% or more lipid present in stratum corneum. In the latter tissue, increases in the permeability rates of the polar n-alkanols are exaggerated after lipid extraction with the chloroform/methanol (Blank et al 1967) as extraction of lipids results in a functionally porous matrix. The removal of lipid from the human nail plate in the present studies caused an increase in the penetration rates of water, methanol, ethanol and butanol but reduced the rates of transfer of the C_{10} and C_{12} compounds (Table 1). The presence of only minor amounts of lipid in the nail plate, a significant portion of which must be carried over as lipid from former cell membranes, suggests that this structure should be far less sensitive than the stratum corneum to the effects of extraction, as is observed with the lower chain length homologues. On the other hand, the high permeability coefficient of n-decanol

Table 1. Permeability coefficients of water and n-alkanols across normal and delipidized nail.

	Permeability coefficients[a] ($\times 10^3$) cm h^{-1}	
Permeant	Normal nail	Delipidized nail
Water	16·5 ± 5·9 (6)	22·4 ± 3·6 (5)
Methanol	5·6 ± 1·2 (26)	10·5 ± 2·3 (20)[b]
Ethanol	5·8 ± 3·1 (8)	6·9 ± 0·3 (5)
Butanol	0·6 ± 0·3 (4)	2·6 ± 0·8 (5)[b]
Decanol	2·5 ± 1·7 (10)	0·5 ± 0·05 (5)[b]

[a] Data include standard deviation and ( ) number of experiments.
[b] Indicates significant differences ($P < 0.005$).

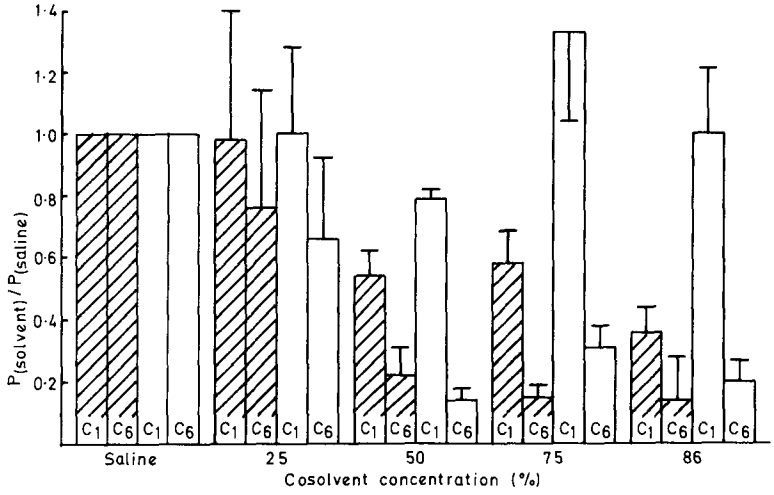

FIG. 2. Effect of co-solvent concentration on the nail permeation of methanol (C_1) and n-hexanol (C_6). The data is normalized with respect to the permeability coefficient in saline. Error bars indicate standard deviation (n = 4). Hatched columns DMSO; open columns isopropyl alcohol.

appears to be dependent on the presence of lipid in the barrier as a six-fold decrease in the permeation rate of decanol across the nail is evident following delipidization. This observation is consistent with a minor lipid pathway which becomes rate-controlling for molecules as hydrophobic as decanol. The selectivity of effects with decanol and dodecanol under all conditions indicates the lipid is organized in a way favourable to their inclusion.

In Fig. 2, data are provided which indicate that DMSO as a vehicle acts to reduce the mass transfer coefficients of methanol, a very polar solute, and also n-hexanol, an alkanol which is hydrophobic and practically insoluble in water. The influence on hexanol is decidedly greater. Studies by Kurihara (T. Kurihara, personal communication) in these laboratories have shown, through partial vapour pressures, that at 2% v/v, the thermodynamic activity of methanol is gradually, systematically increased as the percentage of DMSO increases in binary DMSO–water mixtures. The systematically declining permeability coefficient of methanol with increasing DMSO concentration could therefore not be thermodynamic and due to declining partitioning tendencies. However, on the same strict thermodynamic grounds, the permeability coefficient of hexanol should rapidly become extremely small because its activity drops precipitously with increasing DMSO. It does not. The permeability coefficient is suppressed not much more than five-fold even with 86% DMSO in the solvent medium. This magnitude of effect is the same as when hexanol is applied in its neat state relative to a highly dilute aqueous solution, again suggesting hydration as the facilitating mechanism. The permeation behaviour of hexanol from isopropanol–water systems is similar to its behaviour from DMSO–water systems. The methanol permeability coefficient is unaltered by the presence of isopropanol (Fig. 2). Thus methanol remains the curious exception to the general alkanol behaviour. The effect on hexanol of substituting isopropanol for water in the vehicle is some five-fold, there being a consistent fall in rate when the applied medium contained little or no water.

The enhanced permeability of stratum corneum as the result of contact with strongly interacting solvents such as DMSO, is in sharp contrast to the effects of DMSO on nail (MacGregor 1967; Montes et al 1967; Allenby et al 1969; Embery & Dugard 1971; Chandrasekaran et al 1977; Jones private communication). Having far less lipid and a much more tightly woven intracellular structure (Forslind & Thyresson 1975), the nail, in all likelihood, is much less affected by alteration caused by lipid extraction and there is no indication it undergoes delamination. The nail apparently is also incapable of absorbing much DMSO (Kligman 1965). The crystallinity of nail proteins is different from that of the stratum corneum. All these factors set the nail apart from the stratum corneum with respect to barrier properties in general and the DMSO actions in particular. The inability of DMSO to enhance chemical penetration of hydrophilic or lipophilic permeants into the keratinous matrix of ovine hoof

material (Malecki & McCausland 1982), a nail plate-like structure, is a related observation pointing to differences in hard keratin structures and stratum corneum. It thus seems clear that solvents which tend to promote diffusion through the stratum corneum may be of little use as facilitators of nail plate permeation.

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