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Percutaneous penetration of multipolar ions: evidence for porous transport

Malgorzata Sznitowska [a,b], Bret Berner [c], Howard I. Maibach [a,*]

[a] *University of California, Department of Dermatology, San Francisco, CA 94143, USA*
[b] *Department of Pharmaceutical Technology, School of Medicine, Al. Hallera 107, 80-416 Gdansk, Poland*
[c] *Ciba-Geigy Corporation, 444 Saw Mill River Rd, Ardsley, NY 10502, USA*

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Abstract

The in vitro percutaneous absorption from ethanolic and hypertonic solutions of multipolar ions, aspartic acid, lysine and histidine, was studied. At constant concentration, no increase in the fluxes in the presence of ethanol was observed in spite of the decreased solubility of the amino acids in these solutions compared to water, and this is not consistent with a partitioning mechanism of transport. Porous transport is proposed as the mechanism, and the decreased rate of penetration under hypertonic conditions is also consistent with diffusion of such ionized species through pores in stratum corneum.

Keywords: Skin permeation; Transdermal delivery; Amino acid; Porous transport

1. Introduction

The passive transport of ions across the stratum corneum is still a subject of considerable controversy. However, the percutaneous penetration of inorganic ions has been extensively investigated (Tregear, 1966) and intercellular deposition in stratum corneum has been demonstrated (Wahlberg, 1968; Boddé et al., 1991). Diffusion of ions across the stratum corneum has been suggested to occur through separate pores (Berner et al., 1987; Ghanem et al., 1992), but this theory of porous transport is not fully accepted due to the paucity of evidence (Guy et al., 1988).

Membrane permeation may occur through two basic mechanisms: (1) partitioning and (2) porous transport. While the rate of permeation for a partitioning process is related to the activity of the penetrant in the donor solution, the rate of transport through pores is related to the concentration in the donor phase. Mazzenga et al. (1992) showed a linear correlation of the steady-state flux across epidermis, J_{ss}, with the solubility in the vehicle for the series of zwitterions and their salts. This observation is consistent with a porous model for transport. Dinh et al. (1993) interpreted the equilibrium and steady-state resistances of skin undergoing iontophoresis in terms of convective transport through charged pores. In

* Corresponding author. University of California, Department of Dermatology, Surge 110, San Francisco, CA 94143–0989, USA.

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the zero current limit, this model reduces to diffusive transport through charged pores with a porosity of 10^{-7}, a pore radius of 25 Å, and a surface charge of -0.05 C/m^2.

Amino acids are good models to investigate permeation of skin by organic ions, because, as multipolar ions, these compounds possess charged groups throughout the entire range of pH examined, including at the isoelectric point. In an earlier report (Sznitowska et al., 1993), the in vitro transport of lysine, aspartic acid and histidine through human skin was shown to be independent of the degree of ionization, and this is also consistent with a porous mechanism of transport. However, the diffusion constants ($3–6 \times 10^{-11}$ cm^2 s^{-1}) were smaller than might be expected for porous transport.

In the current investigation, in vitro skin penetration by the same amino acids from ethanolic or hypertonic solutions has been studied. Ethanol influences the rate of penetration if either porous or partitioning transport occurs, but in a different manner. Provided that salting in and out effects are negligible, hypertonic conditions should affect only diffusion through pores. The experiments were performed then to differentiate between the two possible mechanisms of penetration.

2. Materials and methods

The materials and methods for the in vitro skin permeation and analytical studies have been presented in detail in an earlier article (Sznitowska et al., 1993). Briefly, ^{3}H and ^{14}C-labelled amino acids, L-aspartic acid, L-histidine and L-lysine (NEN Research Products, DuPont Co., Boston, MA) were used. Care was taken to separate in situ formed tritiated water from the tritium-labelled amino acids using the previously described ion-exchange columns (Sznitowska et al., 1993). Soluene® 350 was purchased from Packard Instrument Co. (Downers Grove, IL). Quantum Chemical Co. (Milwaukee, WI) was the source of dehydrated ethyl alcohol (USP) and d-sorbitol was purchased from Aldrich Chemical Co. (Milwaukee, WI).

To study the effect of ethanol on percutaneous absorption 30% (v/v) ethanol was used as a vehicle. The concentrations of histidine and aspartic acid in the ethanolic solutions were chosen to be close to saturation: 0.1% (w/v) solution of histidine and 0.03% (w/v) of aspartic acid were prepared. The apparent pH values of the resulting solutions were 6.2 and 3.2 for histidine and aspartic acid, respectively. For the reference experiments, aqueous solutions of the same concentrations were prepared and the pH of the histidine solution was 7.3 and that of the aspartic acid was 3.4. For the studies with varying osmotic gradients, the following aqueous solutions of amino acids were prepared: 1.0% (w/v) histidine at pH 5.0 and 7.3 and 1.0% (w/v) lysine at pH 7.3 and 8.9 (50–100 mOsm/l), while hypertonic solutions of lysine and histidine were prepared using 0.45 mol/l NaCl solution or 0.9 mol/l sorbitol solution (900–1000 mOsm/l). Sorbitol was also selected to avoid salt effects on the solubility of the amino acids. Gentamicin sulfate was added to all solutions of amino acids in the amount 0.5 mg/ml. Radiolabelled amino acids were dissolved in these solutions to provide approx. 10 μCi/ml.

Dermatomed human cadaver skin (approx. 500 μm thick) from three donors was used (aged 28–38 years). The donor solutions were applied in the amount of 300 μl. The receiver fluid was isotonic saline and the permeation studies were performed for 86 h as described elsewhere (Sznitowska et al., 1993).

To study partitioning into epidermis and dermis, these layers were mechanically separated at the end of the study, solubilized overnight at 37° C in 2 ml of Soluene® 350, and then 80 l of 80% acetic acid was added to reduce the chemiluminescence. After 2 days 10 ml Ultima Gold™ Scintillation Cocktail (Packard, Downers Grove, IL) was added and the amount of radioactivity was measured.

Student's *t*-test was used for statistical analyses of the data.

3. Results

The fluxes of histidine and lysine from 1.0% aqueous solutions are in agreement with those

Table 1
Penetration of amino acids from ethanolic solutions (mean ± SD, $n = 6$)

Parameter	Histidine		Aspartic acid	
	Water	30% ethanol	Water	30% ethanol
J_{ss} ($\mu g\ cm^{-2}\ h^{-1}$)	0.022 ± 0.014	0.013 ± 0.01	0.022 ± 0.003	0.001 ± 0.0005
K_p ($\times 10^{-8}$) ($cm\ s^{-1}$)	0.6	0.36	2.0	0.09
A_{ss} ($\mu g\ cm^{-2}$)	2.07 ± 1.22	2.85 ± 0.65	0.51 ± 0.08	0.75 ± 0.25
A_{epid} ($\mu g\ cm^{-2}$)	1.77 ± 1.02	2.33 ± 0.40	0.44 ± 0.10	0.51 ± 0.4

J_{ss}, steady-state flux; K_p, apparent permeability coefficient; A_{ss}, total amount in the skin; A_{epid}, amount in epidermis.

previously reported (Sznitowska et al., 1993). The apparent permeability coefficients for histidine (Tables 1 and 2) and aspartic acid (Table 1 vs 3×10^{-8} cms^{-1} obtained in the earlier study) from aqueous solutions were at most weakly dependent on the concentration of amino acids in the donor solution, i.e., the permeability is at most 1.8-times smaller for a 10-fold difference in concentration of the permeant.

In Table 1, the parameters characterizing transport from ethanolic solutions are compared with those for penetration from aqueous solutions of the same concentrations. While the flux of histidine from 30% (v/v) ethanol was comparable to that from water, the flux of aspartic acid decreased 20-fold for the ethanolic vehicle. The time lags, T_{lag}, were unaffected by ethanol and remained consistently 20–30 h. The amounts of amino acids in the stratum corneum and epidermis were also unaffected by the presence of ethanol.

Transport parameters from hypertonic solutions for histidine and lysine across skin are presented in Table 2. While the transport of histidine at pH 7.3 was not affected by hypertonic conditions, in vitro permeation of all other multipolar ions across skin was substantially reduced under hypertonic conditions. The amounts of per-

Table 2
Percutaneous penetration of amino acids from water (hypotonic solution) and hypertonic solutions (mean ± SD)

Amino acid/parameter	Vehicle		
	Water	0.45 M NaCl	0.9 M sorbitol
Lysine pH 7.3			
J_{ss} ($\mu g\ cm^{-2}\ h^{-1}$)	0.77 ± 0.25	0.037 ± 0.011	0.035 ± 0.012
A_{ss} ($\mu g\ cm^{-2}$)	59.3 ± 51.4	22.3 ± 21.3	10.6 ± 2.1
A_{epid} ($\mu g\ cm^{-2}$)	48.7 ± 40.7	16.9 ± 18.6	7.2 ± 4.4
n	5	5	5
Lysine pH 8.9			
J_{ss}	4.3 ± 1.6	0.40 ± 0.08	0.12 ± 0.08
A_{ss}	76.2 ± 38.7	21.0 ± 7.0	18.8 ± 8.3
A_{epid}	61.4 ± 37.0	13.6 ± 5.2	16.1 ± 9.4
n	5	5	5
Histidine pH 5.0			
J_{ss}	0.13 ± 0.04	0.074 ± 0.046	0.025 ± 0.01
A_{ss}	7.7 ± 2.3	7.9 ± 3.7	7.4 ± 4.4
A_{epid}	5.3 ± 4.1	6.8 ± 4.0	4.6 ± 3.2
n	6	8	6
Histidine pH 7.3			
J_{ss}	0.30 ± 0.06	0.37 ± 0.09	0.22 ± 0.08
A_{ss}	15.0 ± 1.0	13.4 ± 4.7	8.8 ± 3.3
A_{epid}	12.6 ± 2.2	11.0 ± 3.7	7.2 ± 3.4
n	4	5	6

meants in the dermis and epidermis were not affected by these conditions.

4. Discussion

Ethanol is a potent percutaneous absorption enhancer and the effect of increased penetration has been reported for many drugs covering a wide range of polarity (Ghanem et al., 1992). If a partitioning mechanism of transport predominates for ethanolic solutions (< 30–50% v/v) the solubility of the amino acids in the epidermis should be increased as this drug-partitioning effect appears to operate equally well for neutral and ionizable species (Yum et al., 1994). Moreover, an increase in transport would have been expected as a consequence of the elevated thermodynamic activities of the amino acids at constant concentration in ethanolic solutions compared to water. Such an increase was not observed, however, in the present experiments; the fluxes either decreased or remained the same compared to those from water. While a partitioning mechanism of transport is not consistent with these data, porous transport can be invoked to interpret these results.

At constant concentration, permeation through an uncharged, non-interacting porous network should be unaffected or decreased due to the flux of water into the donor compartment. This is consistent with the observations in Table 1. The addition of surface charge in the pore with a convective back flux of water could further explain the decrease in the presence of ethanol.

The osmolarity of the vehicle should not influence the partitioning of a penetrant into skin. Hypertonic solutions may decrease transport across pores either by shrinking the volume of the pores with dehydration or by the flux of water back into the donor solutions. The results in Table 2 support this hypothesis. The absence of an effect on the flux of histidine from hypertonic solutions at pH 7.3 may relate to a net charge of zero.

Moreover, the effect caused by sorbitol was comparable to or greater than that caused by sodium chloride, and thus, the observed phenomenon appears to be an osmotic effect.

The dramatic decreases in transport of lysine and histidine at pH 5.0 from hypertonic solutions and of aspartic acid from 30% ethanol are typical of changes in permeation through hydrated swollen pores. Such decreases could result from a dependence of pore radius or constriction on hydration, from coupling of permeant transport to the flux of water from the receiver to donor induced by changes in osmolarity, or by changes in the selectivity due to alterations in the Debye layer in the pore. The third interpretation is not consistent, however, with the changes observed with sorbitol. While the absence of an effect on the amounts in stratum corneum and time lag indicate that coupling to an osmotic gradient occurs, the pores' constriction should lead to alteration in either of these parameters.

In conclusion, the comparative permeation from aqueous and ethanolic vehicles with the same concentration of penetrant helps distinguish between a partitioning and a porous mechanism of membrane transport. It is assumed that skin permeation of these selected amino acids, and generally multipolar ions, appears consistent with a porous mechanism of transport. The use of hypertonic media may be useful to prevent skin permeation of toxic ions or multipolar ions.

References

Berner, B. and Cooper, E.R., Models of skin permeability. In Kydonieus, A.F. and Berner, B. (Eds), *Transdermal Delivery of Drugs*, CRC Press, Boca Raton, FL, 1987, Vol.II, pp. 42–43.

Boddé, H.E., Van den Brink, I., Koerten, H.K. and De Haan, F.H.N., Visualization of in vitro percutaneous penetration of mercuric chloride. Transport through intercellular space versus cellular uptake through desmosomes. *J. Controlled Release*, 15 (1991) 227–236.

Dinh, S.M., Luo, C.-W., and Berner, B., Upper and lower limits of human skin electrical resistance in iontophoresis. *AlChE J.*, 39 (1993) 2011–2018.

Ghanem, A.H., Mahmoud, H., Higuchi, W.I., Liu, P. and Good, W.R., The effects of ethanol on the transport of lipophilic and polar permeants across hairless mouse skin: Methods/validation of a novel approach. *Int. J. Pharm.*, 78 (1992) 137–156.

Guy, R.H. and Hadgraft, J., Physicochemical aspects of percutaneous penetration and its enhancement. *Pharm. Res.*, 5 (1988) 753–758.

Mazzenga, G.C., Berner, B. and Jordan, F. The transdermal delivery of zwitterionic drugs: II. The flux of zwitterion salts. *J. Controlled Release*, 20 (1992) 163–170.

Sznitowska, M., Berner, B. and Maibach, H.I. In vitro permeation of human skin to multipolar ions. *Int. J. Pharm.*, 99 (1993) 43–49.

Tregear, R.T., *Physical Functions of Skin*, Academic Press, London, 1966, pp. 53–72.

Wahlberg, J.E., Transepidermal or transfolicular absorption? *Acta Dermatol. Venereol.*, 48 (1968) 336–344.

Yum, S., Lee, E., Taskovich, L. and Theeuwes, F., Permeation enhancement with ethanol: Mechanism of action. In Hsieh, D.S. (Ed.), *Drug Permeation Enhancement. Theory and Applications*, Dekker, New York, 1994, pp. 143–170.