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Transdermal delivery of the tetrapeptide Hisetal (melanotropin (6–9)) and amino acids: their contribution to the elucidation of the existence of an 'aqueous pore' pathway

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Abstract

In order to address the problem of the elucidation of overall valid transdermal transport mechanisms, the permeation data on 20 amino acids and on the tetrapeptide hisetal as well as other literature data about skin permeation were analyzed in the present study. In addition, the iontophoretic as well as passive transport of the tetrapeptide hisetal across human skin was studied using Franz cells. The anode was placed in the side of the diffusion cell facing the epidermis and the cathode in the acceptor compartment. Current densities of 0.5 and 1 mA were applied. After 12 h of passive permeation maximally 1.2 μg hisetal was found in the acceptor compartment. After 2 h of iontophoretic delivery applying a constant current of 0.5 mA, 18.8 μg hisetal was detected while the application of a constant current of 1 mA for 2 h resulted in 28.7 μg hisetal in the acceptor compartment. Consequently, iontophoretic treatment increased the permeation rate of hisetal by a factor of 30. In comparison to the use of enhancers, iontophoretic treatment is much more effective. Facilitated permeation by enhancer treatment increased the permeation rate of hisetal across human skin maximally by a factor of 6. The results of the analysis of literature data, of our previous work on amino acids and the tetrapeptide hisetal, and of the present iontophoretic study show that the skin does not act as a simple lipoid barrier. The observation that increasing hydrophobicity did not correlate with increasing permeability of these amino acids as well as the finding that no relationship between the molecular weights of the amino acids and the peptide was observable led to the conclusion that amino acids and peptides appear to use another pathway through the skin. These findings and the fact that iontophoretic treatment is much more effective than enhancer treatment clearly seem to indicate that amino acids and the peptide permeate the skin mainly through water filled pores.

Key words: Melanotropin (6–9); Tetrapeptide; Human skin; Iontophoresis; Skin permeability; Stratum corneum; Partition coefficient; Molecular weight; Pore pathway; Renkin factor

1. Introduction

To date, the skin's barrier function and the overall transport mechanism through the skin have remained incompletely understood. In par-

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ticular, the pathway of very polar compounds is quite often among the most critical topics in discussions concerning the transport mechanisms. In connection with the above-mentioned compounds, two different hypotheses have been postulated. There is one large group of researchers (Scheuplein, 1965, 1967; Flynn and Yalkowsky, 1972; Ackermann and Flynn, 1987) who support the theory that very polar substances traverse the lipophilic stratum corneum via 'aqueous pores'. The other theory is that, regardless of the physicochemical properties of the compounds, the transport mechanism can be fully characterised as permeation via the stratum corneum lipids (Potts and Guy, 1992).

The objective of the present paper was to discuss our own data as well as those from other research groups in order to gain meaningful insights and support for the possibility of the existence of a polar pathway.

In order to further substantiate this support, preliminary iontophoresis experiments with a tetrapeptide, hisetal (melanotropin (6–9)) through human skin, were also performed.

2. Material and methods

Hisetal, α-MSH (6–9), was purchased from Bachem AG (Bubendorf, Switzerland). The amino acid structure of the tetrapeptide (Mol. Wt = 764; pI = 10.7–10.8) is H-His-Phe-Arg-Trp-OH. The peptide was used in the form of an acetate salt. The peptide content was 84.3%. Phosphate-buffered saline (PBS) pH 7.4 was produced using analytical grade chemicals obtained from Merck (Darmstadt, Germany).

Human skin was obtained from a hospital in Frankfurt (Germany). Breast skin of a 35 year old woman was used. The epidermis was separated from the dermis by a heat separation technique. Therefore, the skin pieces were immersed in water at 60°C for 120 s. Afterwards, the stratum corneum/viable epidermis sheets were carefully separated from the dermis and stored at −20°C until experiments were carried out.

2.1. Iontophoretic experiments

Human skin was mounted on the top of the Franz cell with the viable epidermis facing the acceptor compartment. The cell was made of glass. The acceptor compartment had a volume of 1.3 ml. The surface area of the membrane of the diffusion cell was 0.8 cm^2. The cell was maintained at a temperature of 37 ± 1°C by an external water-jacket. The experimental settings and the cell are shown in Fig. 1.

In the first set of experiments, 100 μl of hisetal (1% in buffer solution) was applied on the epidermis. The acceptor chamber was charged with PBS buffer. A pair of platinum electrodes was used, where the anode was placed on the top of the skin while the cathode was placed in the acceptor compartment. Immediately after filling the acceptor compartment a sample was drawn to determine the initial concentration. A period of passive permeation of hisetal followed. After 12 h, a sample was again taken to determine the amount of passive permeation. Subsequently, the acceptor compartment was evacuated with a syringe and replaced with PBS buffer.

A second set of experiments was initiated by connecting the electrodes to a constant current power supply. Iontophoretic permeation was determined by passing a constant current of 0.5 mA across the skin for 2 h. After 2 h, a sample was removed, and the acceptor compartment was evacuated again and refilled with PBS buffer.

A third set of experiments was carried out. This time the power supply was set to provide a constant current of 1 mA for 2 h.

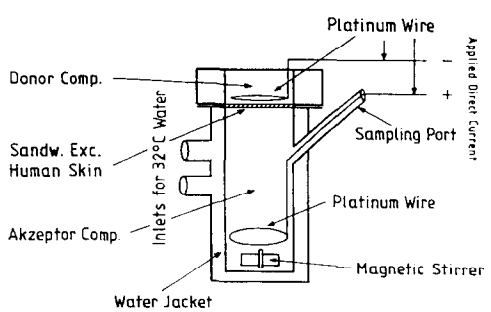

Fig. 1. Schematic representation of iontophoretic system.

The concentration of hisetal in the samples from the diffusion experiments was determined according to a previously described high-performance liquid chromatographic (HPLC) procedure (Ruland et al., 1994a)

3. Results

Fig. 2 shows the skin permeation rate of hisetal under iontophoretic treatment compared to transport by passive diffusion. After 12 h of passive permeation maximally 1.2 μg hisetal was detectable. This corresponds to the passive permeation rate of hisetal across human skin in part II of the study (Ruhland et al., 1994b). The permeation rate with iontophoresis was significantly enhanced. The extent of enhancement in skin permeation rate increased with increasing current density. Although the means from the two different experimental settings do not differ statistically from each other, the results of the single experiments point to this conclusion. At a current density of 0.5 mA, 18.8 μg hisetal was found in the acceptor compartment, whereas 28.7 μg hisetal was detected after applying a voltage of 1 mA. These investigations confirmed previously found passive permeation rates of the tetrapeptide hisetal across human skin. Iontophoretic treatment increased the skin permeation rate of hisetal by a factor of 30. This enhancement factor is significantly greater than those determined during enhancer treatment (Ruland et al., 1994b).

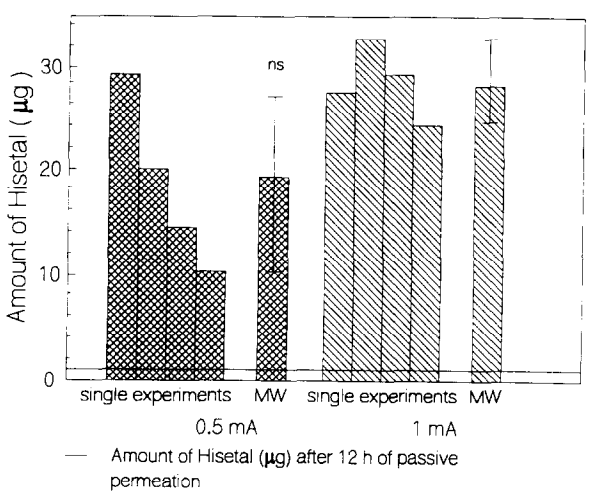

Fig. 2. Amount of hisetal in the acceptor compartment after 2 h iontophoresis at current densities of 0.5 and 1 mA, respectively. $n = 4$; mean ± SD; ns, not significant; * $p < 0.05$, Student's t-test against the data of the experimental setting at a current density of 1 mA.

4. Dicussion

An inviolate rule of diffusion is that molecules follow the path of least diffusional resistance. This path is determined by the physicochemical nature of the membrane in connection with the physicochemical characteristics of the diffusing molecules. Growing knowledge of the physicochemical composition of the stratum corneum lipids as well as results obtained by differential scanning calorimetry (DSC), infrared spectrometry (IR) and Fourier transform spectrometry (FTIR) studies indicate a correlation between lipid structure and barrier function.

Systematic permeation studies with alkyl homologs (Scheuplein, 1965, 1967, 1976; Blank et al., 1967; Behl et al., 1980; Durrheim et al., 1980; Flynn et al., 1981) and alkyl p-aminobenzoates (Flynn and Yalkowsky, 1972) demonstrate the importance of the lipophilicity of a substance. A plot of permeability coefficient vs partition coefficient results in a sigmoidal curve, indicating a linear correlation over a broad range. However, the sigmoidal character also provides evidence of an alternative pathway without an o/w partitioning dependency.

Fig. 3 shows a plot of the permeability coefficients of amino acids (Ruland and Kreuter, 1992) vs the log of the o/w partition coefficients. The plot clearly demonstrates that there is no correlation between increasing lipophilicity and permeability coefficient. Moreover, data from the above-mentioned study do not provide any evidence that the unionized amino acid leads to greater permeation coefficients. All these results, therefore, indicate that skin does not act as a simple lipoidal barrier.

A logical alternative consequence of the above-mentioned facts would be transport

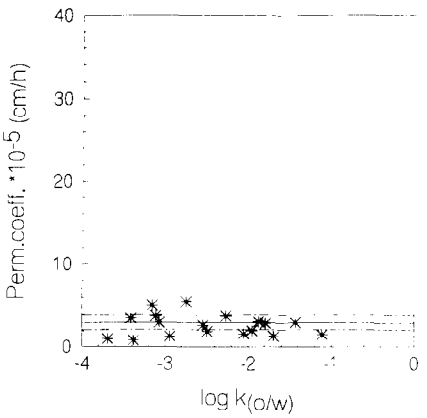

Fig. 3. Plot of the permeability coefficients of the amino acids (measured at pH 7.4 across hairless mouse skin) vs log $k_{(o/w)}$ ($n = 3$–5).

through water filled pores. Several studies with porous artificial membranes demonstrate that transport through water filled pores is independent of the state of ionization as well as of o/w partition coefficients. In this context, a very interesting study was performed by Hatanaka et al. (1992). This study clearly demonstrated that diffusion across a silicon membrane was to a large extent dependent upon the partition coefficient of the molecule whereas diffusion through porous membranes was relatively independent of the partition coefficient. An interesting study on diffusion through pores was reported by Ho et al. (1983). The latter authors defined the permeability coefficient P_p through water filled pores as:

$$P_p = \frac{\epsilon D_{aq} F}{\tau L} \quad (1)$$

where ϵ represents the volume fraction of aqueous pores, D_{aq} the diffusivity in water, L the thickness of the membrane, τ the tortuosity factor (≤ 1) and F the Renkin filtration factor. The effective diffusivity of the molecule in the aqueous pores is defined by the product of the unrestricted diffusivity in bulk water and the filtration factor, namely, $D_{aq}F$. The filtration factor accounts for the frictional resistance encountered by the molecule at the entrances and within the channel of the pores and, for cylindrical pores, is expressed by:

$$F = (1 - r_s/R_p)^2\left[-2.104(r_s/R_p) + 2.09(r_s/R_p)^3 - 0.95(r_s/R_p)^5\right] \quad (2)$$

where r_s is the molecular radius and R_p denotes the pore radius. The Renkin equation is based on permeability measurements with dialysis membranes and is widely used to estimate the permeability properties of biological membranes.

For water diffusion, a plot of D vs molecular weight results in a straight, approximately horizontal line which indicates only a slight dependence on the molecular weight. In contrast, for diffusion through more structured membranes like the stratum corneum, the plot should show a straight line decreasing with increasing molecular weight. At the same time, the degree of dependency should correlate with pore size.

Fig. 4 shows a plot of the permeability coefficients of amino acids and the tetrapeptide hisetal vs molecular weight. The plot clearly demonstrates that the permeability is independent of the molecular weight of the substance. Furthermore, the plot clearly shows that the Renkin factor does not influence permeation, which is indicative of relative large pores within the skin.

In contrast to the above-mentioned theory, Potts and Guy (1992) postulated skin permeation

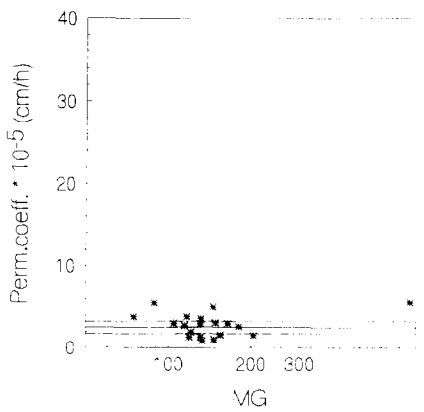

Fig. 4. Plot of the permeability coefficients of the amino acids and hisetal (measured at pH 7.4 across hairless mouse skin) vs permeant molecular weight ($n = 3$–5).

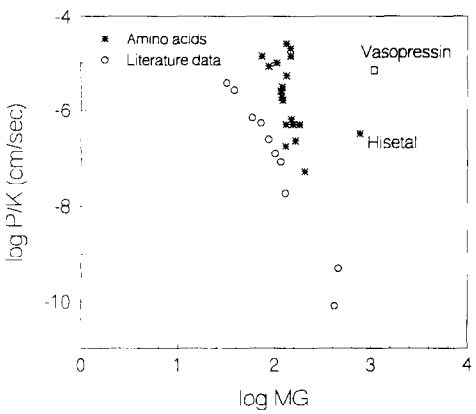

Fig. 5. Plot of permeant diffusivity expressed by normalisation of P by K vs permeant molecular weight. Data from Durrheim et al. (1980), Banerjee and Ritschel (1989) and Smith (1982) were plotted as well as data on the amino acids and hisetal from our studies. All the data were determined using hairless mouse skin as a diffusion membrane.

without the existence of aqueous pores within the skin. Furthermore, they concluded that their model of skin permeation is consistent with transport through lipid lamellae and that the lipid properties are sufficient to explain the permeability of the stratum corneum. Among other things, they substantiated their theory with an interesting plot in which an inverse dependency of some substances on the molecular volume was presented, in accordance with the following expression:

$$P = \frac{KD}{h} \tag{3}$$

where P is the permeability coefficient, K denotes the partition coefficient, D is the permeant diffusivity and h represents the diffusion path length.

In Fig. 5 data from other research groups as well as those of our study employing all natural amino acids including the tetrapeptide hisetal are plotted in a similar manner to that of Potts and Guy (1992), who depicted their results in the form of a plot of log P/K vs log molecular weight (MG). An alteration was involved in the present plot in that Potts and Guy used the molecular volume instead of the molecular weight. Potts and Guy explained the permeability of small, polar compounds on the basis of their molecular volume. With amino acids the molecular volume is closely related to the molecular weight.

In contrast to the findings of Potts and Guy, Fig. 5 depicts no linear relation between P/K and MG. Fig. 5 clearly indicates that the molecular weights of amino acids and small peptides do not correlate over the entire range of molecular weights with the diffusion coefficient expressed as P/K, therefore, invoking the existence of an aqueous pore pathway.

Furthermore, the above investigations concerning the iontophoretic delivery of hisetal across human skin also support the existence of aqueous pores within the skin. As mentioned, the permeation rate of hisetal across human skin was significantly enhanced by a factor of about 30. Consequently, the permeation rates of hisetal were significantly greater with iontophoretic treatment rather than with enhancer treatment (maximal factor 6).

However, if there were only an aqueous pathway for the amino acids or peptides, one would expect no relationship to exist between log P/K and log MG for their transport. In fact, as demonstrated by Fig. 5, at lower log P/K the data converge towards the data line of Potts and Guy. Further evidence for transport via a lipoidal pathway lies in the ability of enhancers to effect an increase in the permeation of amino acids and of hisetal through skin. Taken together, these observations suggest that both, aqueous pore and lipoidal pathways, contribute to the diffusion of amino acids and small peptides through the skin allthough the aqueous pathway appears to prevail with these compounds.

5. Conclusion

The results of the present and former investigations combined with data from other research groups indicate that the skin does not act as a simple lipoidal barrier. The transdermal permeability of amino acids and the tetrapeptide hisetal does not depend on their molecular weights. Similar results were reported in studies with porous artificial membranes (Hatanaka et al., 1990). Fur-

thermore, the plot of D expressed as P/K vs molecular weight (Fig. 5) indicates relatively large pores within the skin.

Unlike the findings of Potts and Guy (1992), the above-mentioned plot (Fig. 5) in which data from polar, high molecular weight substances (hisetal, vasopressin) were also included, does not show a linear correlation between D and MG.

Last but not least, iontophoretic treatment of hisetal across human skin resulted in graeter permeation rates than enhancer treatment. The mechanism of iontophoresis mainly works on the basis of transport through water filled pores. For this reason, the above-mentioned findings also support the existence of pores within the skin.

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