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Transdermal delivery of the tetrapeptide Hisetal (melanotropin (6–9)): II. Effect of various penetration enhancers. In vitro study across human skin

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Summary

The percutaneous absorption of the tetrapeptide hisetal as well as the effect of various penetration enhancers on the permeation of hisetal across human skin was evaluated by in vitro methods in Franz cells. The passive permeability coefficient for hisetal was found to be 0.93×10^{-5} cm h^{-1}. In comparison to the permeation across hairless mouse skin (findings of part I (Ruland et al., *Int. J. Pharm.*, 101 (1994) 57–61) the permeability coefficient was decreased by a factor of 6. Enhancer treatment led to an increase in permeability by a factor of maximally 6 (OA). The relatively new permeation enhancers DDAA and DAIPD were found to increase the permeation of hisetal to similar extents as Azone®. In order to show that the decreased enhancer effects were not due to the experimental design, a second set of investigations was carried out. Whereas drug and enhancer were applied simultaneously during the first set, in the second set of investigations the human skin was pretreated with neat enhancer for 3 h. The results from this second set did not differ significantly from those of the first set. Consequently, these results combined with the findings of part I (hairless mouse skin penetration) clearly demonstrated that hairless mouse skin is influenced by enhancer treatment in an exaggerated manner.

Introduction

In part I (Ruland et al., 1994) of these investigations, the transdermal permeation of the tetrapeptide hisetal across hairless mouse skin was evaluated. By in vitro methods the passive permeation as well as the influence of the penetration enhancers oleic acid, *N*-dodecylazacycloheptan-2-one, Azone®, and dodecyl *N,N*-dimethylamino acetate (DDAA) on the permeation of hisetal was investigated in side-by-side diffusion cells (infinite dose technique). The permeability coefficient of hisetal was found to be 5.58×10^{-5} cm h^{-1}. It was also shown that enhancer treatment resulted in a significant increase in permeation of hisetal (by a factor of 26 in the case of oleic acid). Nevertheless, a number of authors, for example,

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Bond and Barry (1988), Agrawala and Ritschel (1988), Hou and Flynn (1989), and Rigg and Barry (1990), have demonstrated that hairless mouse skin shows exaggerated effects upon treatment with penetration enhancers. Furthermore, Dawson et al. (1988) found the permeation of α-MSH to differ widely from species to species.

For these reasons, the present work was carried out in order to investigate the human skin permeability of hisetal. In this context, great store was set by the influence of the above-mentioned penetration enhancers and a further new penetration enhancer, namely, *N,N*-dimethylaminoisopropanol dodecanoate (DAIPD), on human skin. In doing so, the in vitro technique was changed. This time the experiments were carried out in Franz diffusion cells that resemble the in vivo situation more closely. Over the experimental time frame employed in the present study, the concentrations of hisetal on the donor side in buffer solution as well as hisetal in enhancer solution did not decrease significantly. Therefore, calculation of the permeability coefficient was feasible.

Materials and Methods

Chemicals

Hisetal (α-MSH (6–9)) was purchased from Bachem AG (Bubendorf, Schwitzerland). The amino acid structure of the tetrapeptide (Mol. Wt = 764; p*I* = 10.7–10.8) is H-His-Phe-Arg-Trp-OH. The peptide was used in the form of the acetate salt. The peptide content was 84.3%.

Buffer, PBS (phosphate-buffered saline pH 7.4), was prepared to be iso-osmotic using analytical grade chemicals obtained from Merck (Darmstadt, Germany).

Propylene glycol and oleic acid (Fluka AG, Buchs, Schwitzerland), and *N*-dodecylazacycloheptan-2-one (Azone®) (Nelson Research, Irvine, U.S.A.) were used as received. Dodecyl *N,N*-dimethylamino acetate (DDAA) was synthesised as described previously (Wong et al., 1989). Dimethylaminoisopropanol dodecanoate (DAIPD) was synthesized as described by Büyuktemkin et al. (1993).

Diffusion membrane

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. 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. The stratum corneum/viable epidermis sheets were stored at −20°C prior to carrying out experiments.

In vitro skin permeation

Human skin was mounted on the top of the Franz cell with the viable epidermis facing the receptor compartment. The cell was made of glass. The receptor 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 37 ± 1°C by an external water-jacket. Each experiment was repeated three to four times (n = 3–4). The donor vehicle consisted of 50% (v/v) propylene glycol in PBS buffer, to which 5% (v/v) of the respective enhancer was added. The enhancer was fully soluble in the vehicle at this concentration. The enhancer concentration and the vehicle composition were chosen in order to perform the present investigation in the same manner as in previous studies (Ruland and Kreuter, 1992; Ruland et al., 1994). The latter studies demonstrated that significant enhancement effects were achieved by choosing the above-mentioned enhancer concentrations.

In the first set of experiments, 200 μl of one of the donor vehicles (Table 1) containing 9 mg hisetal and the respective enhancer was applied onto the skin. The receptor chamber was charged with PBS buffer. The permeation of hisetal was followed over a period of 48 h.

In order to ensure that the results of the first set of experiments (simultaneous application of enhancer and drug) were not due to experimental artefacts, a second set of experiments were carried out. Human skin was mounted on the top of the Franz cell in the same way as in the first set of experiments. This time 10 μl of the appropriate enhancer alone was applied to the skin for 3 h. Subsequently, the residual enhancer was re-

TABLE 1

Enhancement factors (EF) of the respective penetration enhancers [a] at a concentration of 5% using human skin as a membrane, [b] after pretreatment with 10 μl of neat enhancer for 3 h

Penetration enhancer	Hisetal [a]		Hisetal [b]		
	EF + SF	P [c]	EF + SD	P [c]	P [d]
OA	5.09 ± 3.66	ns	6.29 ± 2.15	s	ns
A	3.30 ± 0.99	s	3.98 ± 0.59	hs	ns
DDAA	3.72 ± 2.26	ns	4.28 ± 0.22	hs	ns
DAIPD	2.90 ± 0.78	hs	4.07 ± 0.14	hs	ns

The enhancement factor is defined as the ratio of the permeability coefficient with penetration enhancer/permeability coefficient without penetration enhancer. $n = 3$; mean ± SD; ns, not significant; s, $p < 0.05$; hs, highly significant, $p < 0.01$. [c] Student's t-test vs the data of hisetal without enhancer treatment. [d] Student's t-test vs the data of hisetal with the respective enhancer used at a concentration of 5%. OA, oleic acid; A, Azone®; DDAA, dodecyl *N,N*-dimethylamino acetate; DAIPD, *N,N*-dimethylaminoisopropanol dodecanoate.

moved from the skin. Then 200 μl of the peptide buffer solution was applied to the skin. The permeation of hisetal was followed again over 48 h.

Analysis

The concentration of hisetal in the samples from the diffusion experiment was determined by a high-performance liquid chromatographic (HPLC) procedure described in detail in part I of these investigations.

The analysis of the data was described in detail previously (Flynn et al., 1974; Durrheim et al., 1980; Ruland and Kreuter, 1991).

Results and Discussion

As mentioned in the Introduction, the present work was carried out in order to investigate the percutaneous absorption of the tetrapeptide hisetal as well as the influence of some penetration enhancers on the permeation of hisetal across human skin. Furthermore, the influence of the design of the experiments was examined. Therefore, in a first set of experiments the effects of the various penetration enhancers on the permeation of the tetrapeptide hisetal through human skin were examined by applying the enhancer in a propylene glycol buffer solution together with the appropriate amount of hisetal. In these investigations, the enhancers were used at only one concentration (5%) in order to determine whether the effects were similar to those evaluated with hairless mouse skin. In the second set of experiments the design was changed. This time the skin was pretreated for 3 h with 10 μl of neat enhancer.

The permeability coefficient for the passive diffusion of hisetal across human skin was found to be 0.93×10^{-5} cm h^{-1}. In comparison to the permeability coefficient across hairless mouse skin (part I) the coefficient was decreased by a factor of 6.

Table 1 shows the influence of the various penetration enhancers on the permeation of hisetal across human skin. None of the investigated enhancers increased the permeation of hisetal across human skin to the same extent as that across hairless mouse skin. However, similarly to the investigations with hairless mouse skin, oleic acid exhibited the strongest permeation increasing effect. DDAA and DAIPD showed similar enhancing effects to that of Azone®. The permeation increasing effects after pretreatment with neat enhancer 3 h prior to application of hisetal in buffer solution led to slightly higher, although not statistically different, permeability coefficients compared to the results obtained after simultaneous application of peptide and enhancer. This clearly demonstrates that both experimental designs lead to similar results. Similar results were also obtained by Hoogstraate et al. (1991). These authors investigated the enhancing effects of *N*-alkylazacycloheptanones on the per-

meation of the peptide (DGAVP) across human skin.

In conclusion, these investigations have provided the passive as well as the enhancer-facilitated permeation rates of the tetrapeptide hisetal across human skin. The permeability coefficient of hisetal was found to be 0.93×10^{-5} cm h^{-1}. As a result of the treatment with enhancer, the permeability coefficient increased maximally by a factor of 6 (OA). It was also shown that the two different experimental designs, pretreatment with enhancer or simultaneous application of enhancer, led to similar results. Combined with the findings of part I (Ruland et al., 1994) these results clearly demonstrate that the permeation of the tetrapeptide hisetal differs from species to species. Human skin is less permeable than hairless mouse skin. Furthermore, it has been shown that the permeation increasing effects of the investigated permeation enhancers are significantly greater for hairless mouse skin.

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