*International Journal of Pharmaceutics*, 101 (1994) 57–61



IJP 03303

# Transdermal delivery of the tetrapeptide hisetal (melanotropin (6–9)). I. Effect of various penetration enhancers: in vitro study across hairless mouse skin

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(Received 26 February 1993)
(Modified version received 9 April 1993)
(Accepted 27 April 1993)

*Key words*: α-MSH(6–9); Melanotropin (6–9); Tetrapeptide; Permeability; Hairless mouse skin; Penetration enhancer; Oleic acid; Azone®; Dodecyl *N,N*-dimethylamino acetate

## Summary

The percutaneous absorption of the tetrapeptide hisetal as well as the effect of various penetration enhancers on the permeation of hisetal across hairless mouse skin was evaluated by in vitro methods in side-by-side diffusion cells (infinite dose technique). Although the molecular weight of the tetrapeptide is about 4-fold higher in comparison to the amino acids, the permeability coefficient of hisetal was found to be in the same order of magnitude as those of the amino acids. ($5.58 \times 10^{-5}$ cm $h^{-1}$). The permeation of hisetal was increased by enhancer treatment with oleic acid (3%) by a factor of 28. The relatively new permeation enhancer DDAA was found to increase the permeation of hisetal in concentrations of 3% to a higher extent (1.5-fold) than Azone® at the same concentration. The mode of action of DDAA could not be determined by these investigations. However, it was shown that the DDAA effects as well as those of the other penetration enhancers were not reversible within 12 h. These findings lead to the assumption that DDAA induces its permeability enhancing effect on the basis of changes in the lipid structure of the stratum corneum similarly to Azone® and oleic acid.

## Introduction

With the recent advances in recombinant DNA technology, the list of available therapeutic protein and peptide agents is expanding rapidly. The difficulties arising from the delivery of these drugs by classical routes of administration have been discussed frequently (Banga and Chien, 1988; Eppstein and Longenecker, 1988; Lee, 1988; Patel, 1989). Therefore, interest in administering peptide and protein drugs via alternative routes is growing. The transdermal delivery of peptides may offer an attractive possibility for several reasons. The main advantages of the transdermal route are the low proteolytic activity of the skin and the possibility to deliver drugs with short half-lives continuously. Recent studies demonstrate the capability of some peptide drugs to

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penetrate across the skin, e.g., thyrotropin-releasing hormone (Burnette and Marrero, 1986), GRF (1–29)-$NH_2$ (Loden and Faijerson, 1988), leuprolide (Meyer et al., 1988), $\alpha$-MSH (Dawson et al., 1988), vasopressin (Banerjee and Ritschel, 1989), des-enkephalin-$\gamma$-endorphin (Boddé et al., 1989), arginine-vasopressin (Lelawongs et al., 1990) and des-glycinamide arginine-vasopressin, (Hoogstraate et al., 1991). However, the amount which can be administered transdermally is quite low. Therefore, it will often be desirable to facilitate the transdermal transport of peptides by the use of penetration enhancers. Oleic acid has been shown to be a potent enhancer in the percutaneous absorption of amino acids (Ruland and Kreuter, 1992). In this study *N*-dodecylazacycloheptan-2-one (Azone®) was also shown to increase significantly the permeation of amino acids through hairless mouse skin. Recently, Azone® has been found to be successful in increasing the percutaneous transport of peptides (Banerjee and Ritschel, 1989; Hoogstraate et al., 1991). Dodecyl *N,N*-dimethylamino acetate (DDAA), a relatively new penetration enhancer, is known to be nontoxic, non-irritating and biodegradable. This enhancer has been found to enhance the transdermal penetration of indomethacin (Wong et al., 1989), clonidine and indomethacin (Fleeker et al., 1989) as well as 5-fluorouracil, indomethacin and propranolol (Hirvonen et al., 1991).

The present work focuses on the transdermal permeation of the tetrapeptide hisetal through hairless mouse skin. After evaluating the passive transdermal permeation rate, the transdermal transport was increased by the use of the above-mentioned penetration enhancers.

Hisetal, better known as $\alpha$-MSH (6–9), is a part of the endogenous hypophysic hormone melanotropin. Nakanishi et al. (1979) have found the sequence His-Phe-Arg-Trp to be the active centre of the hormone. In addition to several different pharmacological effects, hisetal was found to be effective for the treatment of multiple sclerosis. In a large group of patients Kießling (1987) found the tetrapeptide hisetal to be effective after transdermal administration of a solution of this compound. The present work was carried out in order to investigate the hairless mouse skin permeability of hisetal. In these experiments propylene glycol was chosen as a cosolvent for the penetration enhancers because previous experience with this compound showed that it had no influence at all on the permeability of hisetal. In this first part of our study hairless mouse skin was used as the model skin membrane in order to allow a comparison of the permeability data and the enhancer effects with an earlier paper on amino acids (Ruland and Kreuter, 1992). In addition, hairless mouse skin is very frequently used by several authors due to the difficulties in obtaining human skin. In the second part of this study (in preparation) the hairless mouse skin results will be compared to human skin.

## Materials and Methods

### *Chemicals*

Hisetal, $\alpha$-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 form of the acetate salt. The peptide content was 84.3%. Solubility in water was 10 mg/ml.

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).

### *Diffusion membranes*

Female hairless mice, 17–22 weeks of age, strain hr/hr -c3H/Tif Bom (Bommice Bomholtgard Breeding and Research Center Ltd, Ry Denmark) were used for the permeation studies. They were killed with $CO_2$. Both abdominal and dorsal skin was used after careful excision and removal of the subcutaneous tissue.

*Propylene glycol*

Recent studies (Ruland and Kreuter, 1992) have demonstrated that propylene glycol did not have any increasing effect on the permeation of amino acids across hairless mouse skin. Furthermore, the investigations of Barry and Bennett (1987) have shown that human skin was also not influenced by propylene glycol treatment. For these reasons, propylene glycol was used in the present study as a cosolvent in order to increase the solubility of Azone® and DDAA in PBS buffer.

*In vitro skin permeation*

Freshly excised hairless mouse skin was mounted in a two-chamber, side-by-side diffusion cell with the stratum corneum facing the donor half cell. The cell was made of glass. Both donor and receiver compartments had a volume of 1.5 ml. The surface area of the membrane of the diffusion cell was 0.8 $cm^2$. The cell was then immersed in a constant temperature water bath of 37 ± 1°C.

In the initial set of diffusion experiments, the donor chamber was charged with a hisetal solution in PBS buffer (5 mg 1.5 $ml^{-1}$) in order to evaluate the passive permeation rate. In the following sets of diffusion experiments, the donor chamber was filled with one of the vehicles given in Table 1. Such vehicles also contained 5 mg 1.5 $ml^{-1}$ of the peptide. The receiver compartment was filled with 1.5 ml of the phosphate buffer. The contents of the diffusion cell were allowed to equilibrate and were stirred at 100 rpm. A first sample of the donor compartment was used as a close estimation of the initial donor cell concentration. At specified time intervals, samples of 500 μl were withdrawn from the receiver compartment. An equivalent amount of the buffer solution (500 μl) was added to maintain a constant volume. This dilution of the receiver content was taken into account when evaluating the permeation data.

After 9 h, the first set of permeation experiments was terminated. The donor and the receiver compartment were evacuated and rinsed with saline for 12 h in order to remove all residual enhancers and test permeants.

After the 12 h period, a second set of diffusion experiments was initiated with the same skin patches still mounted in the diffusion cells. The permeation procedure was the same as described above with the exception of the donor charge. This time, the content of the donor chamber consisted of phosphate buffer and the appropriate amount of the tetrapeptide. This second set of experiments referred to the so-called 'reversibility experiment' and was carried out for evaluating the reversibility of the enhancer effect.

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

*Analysis*

The concentration of hisetal in the samples from the diffusion experiment was analysed by a high-performance liquid chromatographic (HPLC) procedure. For this analysis a Waters 600 Multisolvent Delivery System with a Waters UV-Detector 481 and a Waters 712 WISP automatic sampler (all from Waters Associates, Eschborn, Germany) and a Shimadzu CR-5A integrator (Shimadzu, Duisburg, Germany) were used with the detection wavelength set at 220 nm. A reverse-phase column (Eurosil Bioselect C18, 4 × 250 mm; Knauer, Bad Homburg, Germany) was used in conjunction with the HPLC system. The solvent system used was a mixture of acetonitrile/trifluoroacetic acid (0.1% v/v) (30%) and water/trifluoroacetic acid (0.1% v/v) (70%), and the flow rate was 1 ml $min^{-1}$. 200 μl of the sample solution was injected into the HPLC ap-

TABLE 1

*Composition of the donor vehicle*

| Vehicle | PBS solution of: | | | |
|---|---|---|---|---|
| | PG | OA | Azone® | DDAA |
| A | 50 | 3 | – | – |
| B | 50 | – | 3 | – |
| C | 50 | – | 5 | – |
| D | 50 | – | – | 3 |
| E | 50 | – | – | 5 |

Values expresssed as % v/v. PG, propylene glycol; OA, oleic acid; DDAA, dodecyl *N,N*-dimethylamino acetate.

paratus. The retention time of hisetal was 6.7 min.

*Stability of hisetal*

Prior to the diffusion experiments the peptide stability under the experimental conditions was examined. The content of hisetal in buffer solution containing small pieces of skin was analysed over a period of 100 h while the solution was stored at 37 ± 1°C. The results demonstrated that hisetal was stable over a period of nearly 40 h.

## Results and Discussion

As mentioned in the Introduction, the present study 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 hairless mouse skin. Hairless mouse skin was used in order to compare the results with those of previous studies with amino acids (Ruland and Kreuter, 1992). Furthermore, the reversibility of the enhancer effects were determined.

Oleic acid was used at only one concentration, namely 3%, because the results of previous studies with amino acids (Ruland and Kreuter, 1992) showed no increased efficacy at higher concentrations. Unlike oleic acid, Azone® has shown a concentration-dependent enhancing effect on the permeation of the amino acids. Therefore, Azone® and DDAA were used at concentrations of 3% as well as 5%.

The permeability coefficient for the passive diffusion of hisetal across hairless mouse skin was found to be $5.58 \times 10^{-5}$ cm $h^{-1}$ This permeability coefficient was of the same order of magnitude as that of the amino acids (Ruland and Kreuter, 1991). Banerjee and Ritschel (1989) determined a similar permeability coefficient ($3.82 \times 10^{-5}$ cm $h^{-1}$) for the nonapeptide vasopressin (Mol. Wt 1084) with hairless mouse skin.

Fig. 1 shows the influence of the various penetration enhancers on the permeation of hisetal across hairless mouse skin. Oleic acid exhibited the strongest permeation increasing effect. Azone® as well as DDAA demonstrated concentration-dependent enhancing effects. The enhancing effect of DDAA for hisetal at the concentration of 3% was significantly greater than that of Azone® at the same concentration, whereas the enhancing effects of DDAA and Azone® at the higher concentration are similar. None of the enhancing effects were reversible within 12 h.

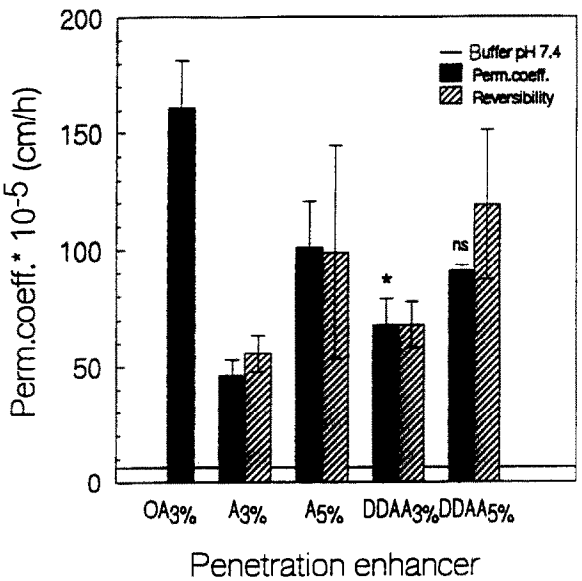


Fig. 1. Influence of the various penetration enhancers on the permeation of hisetal across hairless mouse skin. The straight line depicts the permeation coefficient of the test permeant when phosphate buffer constitutes the medium in the donor compartment. $n = 3$, mean ± SD; ns, not significant; * $P < 0.05$, Student's $t$-test vs the Azone® data at the same concentration. OA, oleic acid; A, Azone®; DDAA, dodecyl *N,N*-dimethylamino acetate.

Wong et al. (1989) and Hirvonen et al. (1991) have also found the enhancing effect of DDAA to be of the same order of magnitude or even of higher order than that of Azone®.

An evaluation of the enhancement factors given in Table 2 provides detailed information about the effects of the investigated penetration enhancers on the permeation of hisetal.

In conclusion, these investigations have confirmed the passive permeation rate of the tetrapeptide hisetal across hairless mouse skin. The permeability coefficient of hisetal was found to be $5.58 \times 10^{-5}$ cm $h^{-1}$. This corresponds to a flux

TABLE 2

*Enhancement factors (EF) of the respective penetration enhancers at different concentrations using hairless mouse skin as a membrane*

| Penetration enhancer (concentration (%)) | Hisetal | |
|---|---|---|
| | EF ± SD | *P* |
| OA (3%) | 28.85 ± 0.64 | hs |
| A (3%) | 8.26 ± 0.11 | hs |
| A (5%) | 18.10 ± 0.8 | hs |
| DDAA (3%) | 12.16 ± 0.7 | hs |
| DDAA (5%) | 16.25 ± 2.18 | hs |

The enhancement factor is defined as the ratio of the permeability coefficient with/without penetration enhancer. ns, not significant; s, significant ($P \ll 0.05$); hs, highly significant ($P \ll 0.01$), OA, oleic acid, A, Azone®; DDAA, dodecyl *N,N*-dimethylamino acetate.

of 0.22 $\mu g\ cm^{-2}\ h^{-1}$. For the treatment of multiple sclerosis hisetal was administered in a dose of 120 $\mu g\ day^{-1}$. Provided that the same permeation rate exists in human skin, the permeation rate of hisetal would not allow the delivery of a sufficient amount of hisetal assuming that the transdermal delivery system has an area of 20 $cm^2$. By incorporating penetration enhancers such as oleic acid (enhancement factor of 28) sufficient drug delivery should be possible.

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