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Dermal delivery of desmopressin acetate using colloidal carrier systems

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Abstract

Recently, the transdermal route has received attention as a promising means to enhance the delivery of drug molecules, particularly peptides, across the skin. In this work, the skin penetration profiles of desmopressin acetate from a colloidal system (water-in-oil microemulsion) and an amphiphilic cream, a standard formulation, were determined using Franz diffusion cells and compared. In the case of the microemulsion, the total percentages of dose obtained from different skin layers (stratum corneum to subcutaneous tissue) were 3.30 ± 0.67, 7.37 ± 2.43 and 15.54 ± 2.72 at 30, 100 and 300 min, respectively. Similarly, 5.19 ± 0.96, 8.04 ± 0.97 and 14.4 ± 5.15% of the dose applied was extracted from the skin treated with the cream. About 6% of the applied dose reached the acceptor compartment from the microemulsion instead of 2% from the cream within 300 min. The concentration of drug that penetrated into the upper layers of the skin was higher from the cream than from the microemulsion at all time intervals. On the other hand, a higher amount of drug was found in the deeper skin layers and in the acceptor compartment from the microemulsion.

Introduction

With the recent advances in synthetic and molecular biology techniques that enable their large-scale production, the interest in peptides as potent drugs has increased dramatically in the last few decades (Lee 1991). However, with very few exceptions, such as small and cyclic peptides (e.g. cyclosporin), most peptide drugs have low oral bioavailabilities (Lee et al 1991). This is mainly attributed to the extensive proteolytic degradation of most peptides by enzymes in the gastrointestinal tract (GI), the poor permeability of the intestinal mucosa to high molecular weight and highly hydrophilic peptides, the insufficient closeness of the drug or delivery system to the absorbing intestinal mucosa and its short residence time at the GI absorption site (Harris & Robinson 1990; Freidman & Amidon 1991; Lee et al 1991). As a result, most new peptide-based drugs are administered parenterally, which is not well accepted by patients, particularly for chronic therapy. The search for alternative routes of drug delivery devoid of such limitations has therefore become essential. Recently, the transdermal route has received attention as a promising means of enhancing the delivery of drug molecules, particularly peptides, across the skin (Morgan et al 1998; Kanikkannan 2002; Robbins et al 2002; Pillai et al 2003; Tao & Desai 2003; Yamamoto et al 2003; Cormier et al 2004). Several techniques, including iontophoresis, electroporation, sonophoresis and the use of microneedle array patch systems, have been tried to accomplish this task. However, most of these methods are harsh and may damage the skin barrier. In addition, the long-term safety of their use, patient compliance with the techniques and the commercial success of the technologies are yet to be demonstrated. Chemical permeation enhancers increase skin permeability for some molecules (Hsu et al 1991; Williams & Barry 1992), but generally require concentrations that cause irritation to be effective.

Microemulsions, which are thermodynamically stable dispersions of oil and water that are stabilized by surfactants and, in some cases, additionally by cosurfactants (de Gennes & Taupin 1982, Langevin 1992), have attracted much interest in recent years because of their great practical importance in terms of their drug delivery potential and interesting physical properties (Malmsten 1998). They are advantageous in that they improve the delivery of

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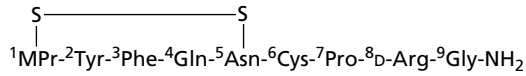
both lipophilic and hydrophilic drugs compared with conventional vehicles, as well as the potential for enhanced absorption due to surfactant-induced permeability changes, depending on the constituents used for the microemulsion vehicle (Boltri et al 1994; Delgado-Charro et al 1997; Dreher et al 1997; Trotta et al 1997). They have been proposed to offer enhanced drug delivery properties for transdermal transport (Trotta et al 1989, 1996) without having most of the disadvantages of the aforementioned techniques.

In this work, in order to evaluate the potential application of microemulsions in the dermal and transdermal delivery of macromolecules, the penetration profile of a model peptide, desmopressin acetate (1-deamino-8-D-arginine vasopressin; DDAVP), from a water-in-oil (w/o) microemulsion was determined. The result was compared with that of a conventional amphiphilic cream. DDAVP is a synthetic analogue of the neurohypophyseal peptide hormone vasopressin that has been developed through systematic structural modifications (Vavra et al 1968). It is a hydrophilic peptide obtained by deamination of cysteine in position 1 of vasopressin, yielding 3-mercaptopropionic acid and by replacement of L- with D-arginine in position 8 (Scheme 1)



Scheme 1

DDAVP is used parenterally and intranasally in the control of haemophilia A, von Willebrand disease, haemorrhage, nocturia enuresis and diabetes insipidus (Cattaneo et al 1990; Shulman et al 1990; Rose & Aledort 1991; Gratz et al 1992; Miller et al 1992). Following oral administration of DDAVP in human volunteers a considerable portion of drug is destroyed in the GI tract. Only 0.7–1% of a given 100 or 200 μg dose appeared in the blood, indicating that the bioavailability of this peptide, although somewhat higher than those of other small therapeutic peptides, is still either equal to or less than 1% (Vilhardt & Lundin 1986), rendering it impractical for simple oral administration. While injectable formulations with doses of 1–20 μg demonstrate better bioavailability, they are poorly suited for routine use.

Materials and Methods

Materials

Methanol and acetonitrile were both HPLC grade and purchased from J. T. Baker (Deventer, The Netherlands). Formic acid and ammonium acetate were obtained from Merck (Darmstadt, Germany). Morphine was purchased from Sigma (Munich, Germany). Isopropyl palmitate (IPP) was purchased from Caesar & Loretz GmbH (Hilden, Germany). Amphiphilic cream DAC, Tagat 02 and Span 80 were from Synopharm GmbH (Barsbüttel, Germany). Desmopressin acetate was kindly donated by Ferring Pharmaceuticals (Kiel, Germany).

Methods

Preparation of the microemulsion of DDAVP

The compositions of the w/o microemulsion and the cream of DDAVP are indicated in Table 1. The DDAVP was first dissolved in water and thoroughly mixed with the mixture of the surfactants, Tagat 02 and Span 80 (3:2). The isopropyl palmitate (IPP) was then added in portions with thorough mixing after each addition. A microemulsion containing all the above components except the drug was also prepared and used as a control.

Preparation of the amphiphilic cream of DDAVP

The amphiphilic cream was warmed in a water bath until melting point and a solution of the DDAVP in water was thoroughly dispersed in the melted base. The amphiphilic cream was used as a control.

Skin penetration study

The penetration of DDAVP through the different layers of human breast skin was determined using Franz diffusion cells (Crown Glass Company, Somerville, New Jersey) (Franz 1975). The studies were performed in triplicate using human breast skin samples from three different patients with a total area of 3.14 cm^2. The skin was obtained after cosmetic surgery. After cleaning with 0.9% sodium chloride solution and removal of the subcutaneous fat, the skin samples were stored at $-20°C$ until required. Before the experiments, the skin samples were thawed and placed onto filter gauze in the diffusion cells. The dermal side of the skin was in contact with the acceptor solution (bidistilled water, 20.0 mL), which was stirred continuously. Twenty microlitres of the microemulsion with or without the drug, 20 mg of the DDAVP cream or 20 mg of the amphiphilic cream DAC was applied on the outer layer of the skin. After incubation at $32 \pm 1°C$, the skin samples were taken at 30, 100 and 300 min. The remaining formulation was wiped with a cotton wool tip and three punch biopsies (each 0.2827 cm^2) were excised from each skin sample. The stratum corneum (SC), epidermis (EP), dermis (DR1, DR2 and DR3) and the subcutaneous tissue (St) were cut in horizontal sections using a cryomicrotome (Jung, Heidelberg, Germany) with the respective thicknesses described in Table 2. Several sections were pooled to one sample to guarantee the

Table 1 Composition of microemulsion and cream

Microemulsion	Cream
DDAVP, 0.75%	DDAVP, 0.75%
Water, 5%	Water, 5%
Tagat 02/Span 80 (3:2), 20%	Amphiphilic cream DAC 2003, 94.25%[a]
IPP, 74.25%	

[a]Amphiphilic cream prepared according to the German Pharmaceutical Compendium.

Table 2 Thickness of skin layers

Skin layer	Number of sections	Thickness per section (μm)	Total thickness (μm)
SC	1	10	10
EP	8	20	160
DR1	5	80	400
DR2	5	80	400
DR3	5	80	400

detection of small drug amounts in the skin. The collected cuts were placed in Eppendorf tubes and extracted with 50% of methanol in bidistilled water as described previously (Getie & Neubert 2004). The cotton wool tips were also extracted in a solvent of similar composition. Samples were also taken from the acceptor compartment (Acc) at the time intervals mentioned above. A constant amount of morphine was added to each of the samples as an internal standard before analysis. The study was approved by the Ethical committee of the Faculty of Medicine, Martin Luther University Halle-Wittenberg, 12 February 2003.

Analytical method

The amount of DDAVP in the different skin layers, the cotton wool tips and the acceptor solution was analysed by the HPLC-MS assay method developed previously (Getie & Neubert 2004). Briefly, the analysis was performed using a Finningan LCQ ion-trap mass spectrometer (ThermoFinnigan, San Jose, CA) coupled with an HPLC pump SpectraSystem P4000 equipped with an autosampler AS 3000 and a membrane degasser. The HPLC column used was a Nucleosil C18 column (CC 125/2, 120-3) purchased from Macherey-Nagel (Düren, Germany). A 0.01% formic acid in a mixture of 1.6 mM ammonium acetate and acetonitrile (33:67, v/v) at a flow rate of 0.2 mL min^{-1} was used as a mobile phase after degassing with helium. The injection volume was 10 μL. The mass spectrometer was operated in an electrospray mode with positive ion detection applying an electrospray voltage of 4.5 kV and a heated capillary temperature of 220°C. The molecular ions at mass to charge ratio (m/z) of 1069.2 and 286.4 for DDAVP and morphine, respectively, were monitored in selected ion monitoring mode and analytical data were acquired by LCQ software. The detection limit and reproducibility of the method are given in our previous article (Getie & Neubert 2004).

Statistical analysis

Results are presented as the mean of three determinations with its associated standard deviation (s.d.). Statistical analysis was performed by two-way analysis of variance and t-test. A probability value of $P < 0.05$ was considered statistically significant.

Results and Discussion

The DDAVP/water/IPP/Tagat 02/Span 80 system yielded a clear and transparent liquid, which did not show any sign of phase separation or droplet growth for at least 1 month on storage at 25°C, as demonstrated by dynamic light scattering (DLS) experiments. The DLS experiments, which were performed on a standard commercial apparatus from ALV-Laser Vertriebsgesellschaft GmbH (Langen, Germany) using a green Nd:YAG DPSS-200 mW laser emitting vertically polarized light at a wavelength of 532 nm, also demonstrated that the hydrodynamic radius of the droplets of our formulation was 4.34 ± 0.12 nm at 25°C (submitted). Accordingly, the system was referred to as a microemulsion.

The amounts of DDAVP that penetrated into the different skin layers and reached the acceptor compartment (expressed as percentage of the applied dose) from the microemulsion as well as the cream formulations at 30, 100 and 300 min are depicted in Figures 1A–C. Each data point indicates the average $\pm$ s.d. of three independent determinations from the punch biopsies that were excised from each of the three skin samples.

It has been observed that the amount of drug that penetrated and remained in the upper layers of the skin was higher from the cream formulation than from the microemulsion at all incubation times. For example, after 300 min of incubation, the amount (percentage of applied dose) of DDAVP observed in the SC from the cream formulation was almost double (3.43 ± 1.0) that obtained from the microemulsion (1.90 ± 0.28). In contrast, the amount of drug reaching the deeper skin layers and the Acc was significantly higher ($P < 0.05$) from the microemulsion than the cream formulation, independent of the incubation time. This is an indication that the two formulations impart different effects on the mode of penetration of the drug through the skin layers.

The concentration profile of DDAVP in the different skin layers from both formulations after 300 min of incubation is presented in Figure 2. A large concentration of the drug was detected in the stratum corneum in both cases. This does not mean, however, that the largest proportion of the drug was contained in the stratum corneum, as its thickness is far less than the deeper layers of the skin (e.g. about 16 times less than that of the viable epidermis). In the case of the microemulsion, the total percentages of the dose obtained from the skin layers (SC, EP, DR1, DR2, DR3 and St) were 3.30 ± 0.67, 7.37 ± 2.43 and 15.54 ± 2.72 at 30, 100 and 300 min., respectively. Similarly, 5.19 ± 0.96, 8.04 ± 0.97, 14.4 ± 5.15% of the dose applied at 30, 100 and 300 min, respectively, were obtained from the skin layers treated with the cream. These results show that there was no significant difference in the total amount of drug that penetrated the skin layers from the two formulations. The percentages of drug that reached the Acc from the microemulsion (2.53 ± 0.98, 4.26 ± 1.83, 6.21 ± 3.21 at 30, 100 and 300 min, respectively) were, however, significantly higher than those for the cream (1.13 ± 0.61, 1.52 ± 0.59, 1.95 ± 0.75 at 30, 100 and 300 min, respectively). It has been also demonstrated that the amount

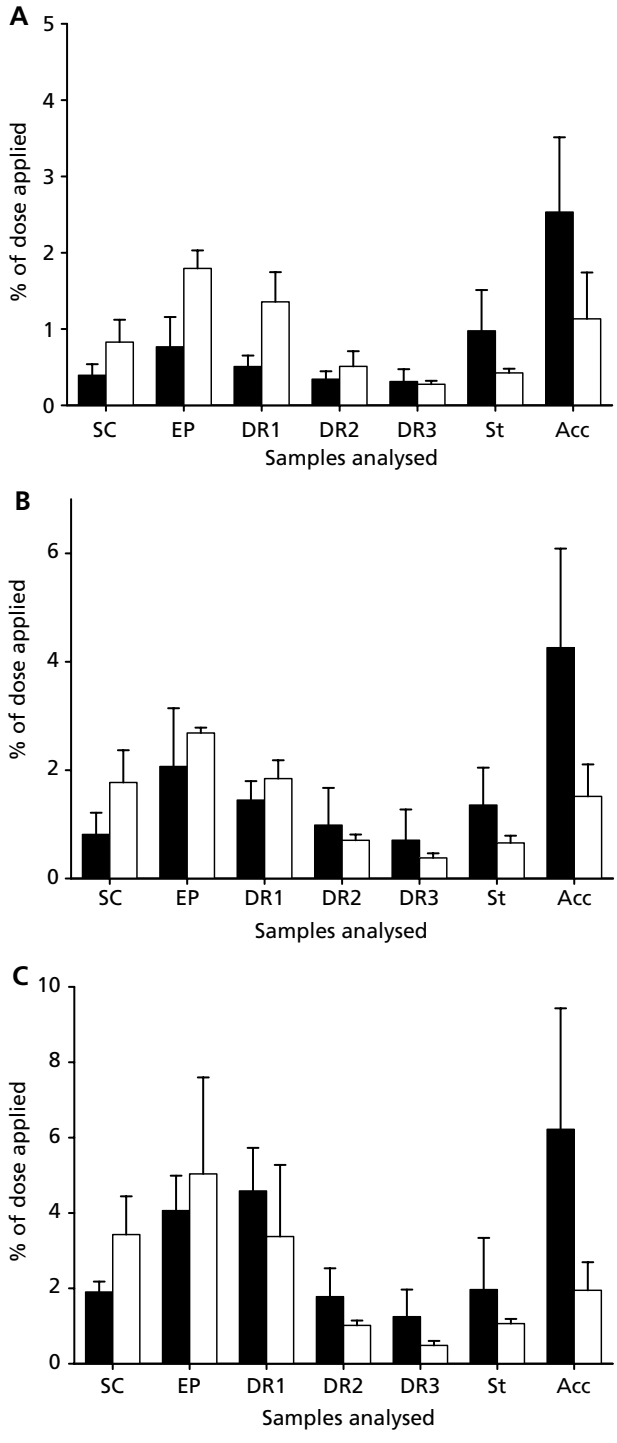


Figure 1 Percentage of DDAVP that penetrated different skin layers and the acceptor compartment from a w/o microemulsion (black) and an amphiphilic cream (white) at 30 (A), 100 (B) and 300 (C) min. SC, stratum corneum; EP, epidermis; DR1–3, dermis; St, subcutaneous tissue; Acc, acceptor compartment.

of DDAVP that penetrated through the skin increases with time and about 15 and 20% of the applied dose penetrated into and through the skin during 300 min from the cream and

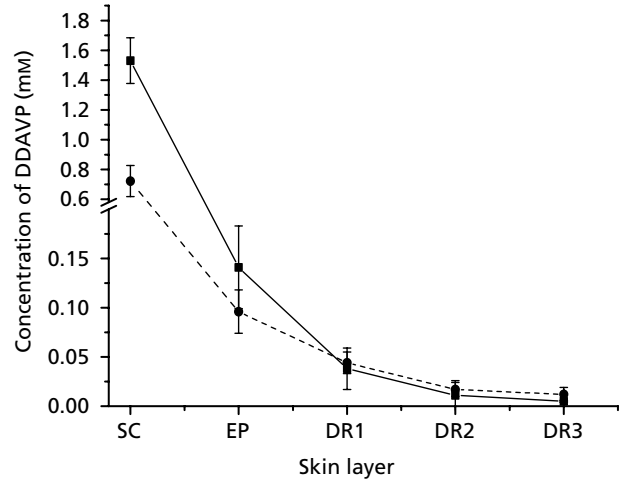


Figure 2 Concentration of DDVAP (mM) in different skin layers from a w/o microemulsion (dashed line) and an amphiphilic cream (solid line) after 300 min of incubation. SC, stratum corneum; EP, epidermis; DR1–3, different dermis sections.

the microemulsion, respectively. Total DDAVP recovery ranged from an average of 70 to 105%.

About 6% of the applied dose, which corresponds to 6.75 μg from the total dose of 112.5 μg, reached the Acc from the microemulsion instead of 2% from the cream formulation within 300 min. Although extrapolation of in-vitro results to the in-vivo situation has to be done with care, compared to the reported 0.7–1% of the same drug that appeared in the blood after a 100 or 200 μg oral dose (Vilhardt & Lundin 1986), the percentage of DDAVP that penetrated into the Acc from the microemulsion formulation is far higher. In addition, it is important to notice that since DDAVP is a low-dose drug taken parenterally in doses ranging from 1 to 20 μg (Fjellestad-Paulsen et al 1993), the amount of the drug that reached the Acc (6.75 μg) is within the therapeutic concentration.

Conclusion

The amount of drug that penetrated the upper layers of the skin was significantly higher from the cream than from the microemulsion at all time intervals. On the other hand, a higher amount of drug was found in the deeper skin layers and in the Acc from the microemulsion. Although the w/o microemulsion does not show an advantage over the cream formulation for dermal delivery of DDAVP, it is of great potential for systemic administration of the drug. Further investigation should be undertaken to enhance and optimize the penetration of the drug from this formulation.

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