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Research Paper

Transdermal Iontophoretic Delivery of Vapreotide Acetate Across Porcine Skin *in Vitro*

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Purpose. The purpose of this study was to evaluate the feasibility of delivering vapreotide, a somatostatin analogue, by transdermal iontophoresis.
Methods. *In vitro* experiments were conducted using dermatomed porcine ear skin and heat-separated epidermis. In addition to quantifying vapreotide transport into and across the skin, the effect of peptide delivery on skin permselectivity was also measured. The influence of (1) current density, (2) pre- and post-treatment of the skin, (3) competitive ions, and (4) inclusion of albumin in the receptor on vapreotide delivery were investigated.
Results. Epidermis proved to be a better model than dermatomed skin for vapreotide transport studies. Despite the susceptibility of vapreotide to enzymatic degradation, a flux of 1.7 $\mu g/cm^2$ per hour was achieved after 7 h of constant current iontophoresis (0.15 mA/cm^2). Post-iontophoretic extraction revealed that, depending on the experimental conditions, 80–300 μg of peptide were bound to the skin. Vapreotide was found to interact with the skin and displayed a current-dependent inhibition of electroosmosis. However, neither the pre-treatment strategies to saturate the putative binding sites nor the post-treatment protocols to displace the bound peptide were effective.
Conclusion. Based on the observed transport rate of vapreotide across porcine epidermis and its clinical pharmacokinetics, therapeutic concentrations should be achievable using a 15-cm^2 patch.

KEY WORDS: electroosmosis; skin barrier; transdermal iontophoresis; vapreotide.

INTRODUCTION

Vapreotide is one of several potent long-acting analogues of somatostatin synthesized for clinical use in the treatment of acromegaly and endocrine tumors of the gastroenteropancreatic system. In addition, it has been successful in the early treatment of esophageal variceal bleeding and has been awarded orphan drug status in the United States (1). These analogues require subcutaneous or intramuscular administration. In an attempt to avoid the parenteral route, more convenient "patient-friendly" routes of drug delivery have been investigated. The results have been mixed: orally administered octreotide (another somatostatin analogue commercialized as Sandostatin®) is hampered by a relatively low bioavailability (2) and because of poor local tolerability, nasal administration is also inconvenient (3). The transdermal route is an attractive alternative to deliver therapeutic drugs (4). The physicochemical properties of peptides (charged; high molecular weight) render them inappropriate for passive transdermal delivery. In contrast, iontophoresis, an electrically assisted drug delivery technology, offers a controlled and noninvasive means of administration (5). The two main transport mechanisms during iontophoresis are electromigration (direct effect of the applied electric field on the charged species) and electroosmosis (convective solvent flow in the anode-to-cathode direction, as a consequence of the skin's net negative charge at physiological pH). Since electrical mobility decreases with molecular weight, it is hypothesized that electroosmosis is the major transport mechanism for peptides (6). It is also known, however, that peptides containing adjacent cationic and lipophilic residues can inhibit electroosmosis, and their own transport, by altering skin permselectivity (7–12). The association of these lipophilic cations with the membrane neutralizes, to varying extents, the intrinsic negative charge of the skin causing a significant reduction in the normal anode-to-cathode electroosmotic flow. Since the degree of inhibition depends on the number of molecules in the skin, it is usually more pronounced at higher applied current densities and at increased peptide concentrations.

The iontophoretic delivery of octreotide has been successfully achieved in rabbits *in vivo* (13). An investigation of the effect of current density and drug concentration on plasma levels of octreotide revealed a decrease in the amount

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ABBREVIATIONS: BSA, bovine serum albumin; EIA, enzyme immunoassay; IF, inhibition factor; RSD, relative standard deviation.

of peptide delivered, with increasing concentration. This might be attributed to an inhibition of electroosmosis, since the -Phe^3-D-Trp^4-Lys^5- sequence in octreotide corresponds to the structural moiety thought to be a prerequisite for this phenomenon (10).

The aim of the current study was to evaluate the feasibility of delivering vapreotide by transdermal iontophoresis. In view of the structural similarity of vapreotide to octreotide, the iontophoretic delivery of vapreotide was expected to affect electroosmotic solvent flow. Hence, in addition to quantifying peptide transport, the magnitude of electroosmotic flow was also determined under the different experimental conditions employed. Experiments were also conducted in the presence of a salt bridge; this allowed the use of lower electrolyte concentrations in the donor compartment, decreasing the number of competing charge carriers and, in theory, increasing peptide delivery efficiency (5). The impact of current density and salt bridge use on both peptide transport and electroosmotic inhibition was measured. Different approaches to modulate the interactions between the peptide and the skin were investigated, including co-iontophoresis with cetrimide, pre- and post-iontophoretic treatment of the skin with Ca^{2+} and propranolol substances capable of interacting with the biomembrane and occupying potential "peptide" binding sites. Vapreotide transport across heat-separated epidermis and dermatomed skin was compared.

MATERIALS AND METHODS

Chemicals

Vapreotide (MW = 1131.4), D-Phe-Cys-Tyr-D-Trp-Lys-Val-Cys-Trp-NH_2, in the form of its acetate salt, was a generous gift from Debiopharm (Debiopharm Galenic Unit, Gland, Switzerland). Acetaminophen was purchased from Fluka (Sigma-Aldrich Chimie, Saint Quentin Fallavier, France). Tris [Tris-(hydroxymethyl) aminomethane], Trizma® hydrochloride, agarose, DL-propranolol, and cetrimide were obtained from Sigma-Aldrich (Sigma-Aldrich Chimie, Saint Quentin Fallavier, France) and bovine serum albumin was purchased from Fluka BioChemika (Sigma-Aldrich Chimie, Saint Quentin Fallavier, France). De-ionized water (resistivity > 18 MOhm/cm^2) was used to prepare all solutions.

Analytical Procedures

Vapreotide extracted from skin samples was quantified using a high-performance liquid chromatography system equipped with a variable wavelength UV detector (Waters Corporation, Milford, MA). The mobile phase, comprising 25% acetonitrile and 75% triethylaminephosphate buffer solution (pH 2.3), was passed through a C18 PartiSphere column (4.6 mm i.d., 12.5 cm long, 5 μm particle size) (Whatman Inc., Florham Park, NJ) maintained at 40°C, at a flow rate of 1 ml/min. The peptide was detected at 280 nm. The RSD of the repeatability was less than 1% and the limit of quantification was 115 ng/ml. Direct competitive enzyme immunoassay (EIA), as a result of its superior sensitivity, was used to quantify the presence of vapreotide in the receptor compartment. This competitive binding assay is based on the relative affinity of (1) the vapreotide (in the sample) and (2) an enzymatic tracer prepared by covalent coupling of vapreotide to an enzyme (acetylcholinesterase), to anti-vapreotide antibodies. The quantification limit corresponded to 100 pg/ml.

Acetaminophen was assayed by high-performance liquid chromatography using a Hypersil BDS C8 column (150 mm × 4.6 mm, Supelco®, Sigma-Aldrich Chimie, Saint Quentin Fallavier, France) maintained at 40°C. The mobile phase (delivered at a flow rate of 1 ml/min) consisted of 92% water and 8% acetonitrile adjusted to pH 3.5 with acetic acid. Acetaminophen was detected by its ultraviolet (UV) absorbance at 243 nm. The RSD of the repeatability was less than 1% and the quantification limit was 22 ng/ml.

Skin Preparation

Porcine ears, obtained from a local abbatoir (Société d'Exploitation d'Abbatage, Annecy, France) within a few hours postmortem, were cleaned under cold running water. The whole skin was removed carefully from the outer region of the ear and separated from the underlying cartilage with a scalpel. The tissue was then either dermatomed (800 μm) or heat-treated to separate the epidermis (14): pieces of fresh full-thickness skin were immersed in water at 58°C for 2 min after which the epidermis was carefully separated from the dermis with a spatula. Pieces of epidermis and dermatomed skin were wrapped individually in Parafilm™ and maintained at −20°C until use and were stored for no longer than a period of 2 months.

Iontophoresis Procedure

The skin was clamped in three-compartment vertical diffusion cells (area: 0.73 cm^2), the design of which has been previously described (15). Unless otherwise stated, vapreotide was dissolved in a 100 mM NaCl solution that was adjusted to pH 5.5 with HCl to produce a 3 mM solution of the peptide; 1 ml of this solution was placed in the donor (anodal) compartment. In addition to vapreotide, the donor compartment always contained 15 mM acetaminophen. The cathodal compartment was filled with 1 ml of 25 mM Tris/Trizma® HCl-buffered (pH 7.4) normal saline. The receptor compartment (~6 ml) was filled with the same electrolyte solution, containing in addition, when stated, 44 g/L of bovine serum albumin (BSA; to mimic physiological conditions) and was stirred magnetically throughout the permeation experiments.

Constant current iontophoresis was used throughout the study. The current, ranging from 0.05 to 0.5 mA/cm^2, was applied for 4–24 h via Ag/AgCl electrodes connected to a power supply (Kepco, Flushing, NY).

When specified, the anode was isolated from the donor solution via a salt bridge (100 mM Tris/Trizma HCl in 3% agarose) to minimize the competition between the peptide and the electrolytes necessary for the anodal reaction. Under these conditions, the anodal compartment contained a solution of 25 mM Tris/Trizma® HCl normal saline buffered to pH 7.4 and the vapreotide in the donor compartment was dissolved in 20 mM Tris/Trizma® HCl (pH 7.4). Schematic

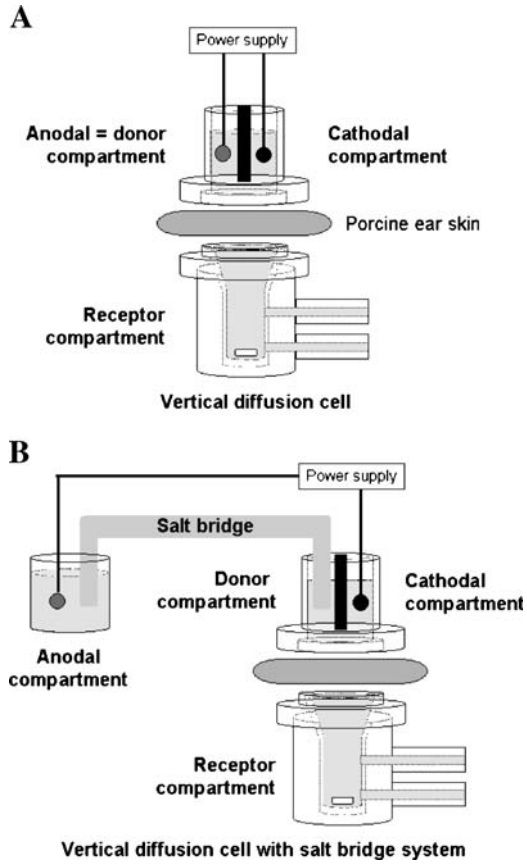

Fig. 1. Schematic representations of the iontophoretic diffusion cell assemblies used in this study.

representations of these two different experimental set-ups are provided in Fig. 1.

Quantifying Electroosmotic Inhibition

Acetaminophen is a neutral hydrophilic compound, with poor passive permeability (~2 nmol/cm^2 per hour); under the influence of an electrical current, this uncharged but polar molecule is driven through the skin predominantly by electroosmosis. Hence, in these studies it was incorporated into the donor formulation (15 mM) to report on convective solvent flow.

For each experiment, an inhibition factor (IF) was calculated using the following equation:

$$IF = [Q_{A\text{-}8h,control}] / [Q_{A\text{-}8h,peptide}] \quad (1)$$

where $Q_{A\text{-}8h,control}$ is the amount of acetaminophen transported into the receptor phase during 8 h of iontophoresis when no peptide was present in the donor solution, and $Q_{A\text{-}8h,peptide}$ is the corresponding quantity when vapreotide was iontophoresed.

Effect of Current Density

To evaluate the effect of current density on electroosmotic inhibition by vapreotide, current densities of 0.15, 0.3, and 0.5 mA/cm^2 were applied for 8 h across dermatomed skin. A solution of 3 mM vapreotide in 20 mM Tris/Trizma® HCl buffered to pH 7.4 was placed in the donor compartment.

Additional experiments were also performed using heat-separated epidermis to assess the effect of current density on the amount of vapreotide transported. Again, a 3 mM vapreotide donor solution was iontophoresed for 8 h.

Vapreotide Accumulation in the Skin

The uptake of vapreotide into dermatomed skin during iontophoresis was also investigated. On terminating current application, the skin sample was rinsed with water, dried on absorbent paper, and then placed in 4 ml of a mixture acetonitrile/water (25:75). After agitation for 14 h, the solution was filtered (0.45 μm regenerated cellulose syringe filter, Alltech, Deerfield, IL) and the amount of peptide quantified.

Skin Metabolism Experiments

The influence of porcine ear skin on the stability of vapreotide was evaluated by filling the receptor compartment with a 5 μM vapreotide solution in 25 mM Tris/Trizma® HCl-buffered (pH 7.4) normal saline. Both dermatomed and epidermal skin samples were evaluated separately. The anodal and cathodal compartments were filled with 1 ml of 25 mM Tris/Trizma® HCl-buffered (pH 7.4) normal saline. A current of 0.15 mA/cm^2 was applied for 8 h and the percentage of intact peptide was calculated as follows:

$$\text{Intact peptide}(\%) = \frac{\text{Final amount of peptide}}{\text{Initial amount of peptide}} \times 100\% \quad (2)$$

Modifying Interactions Between Vapreotide and the Skin

Co-Iontophoresis

One milliliter of 4.4 mM vapreotide in 20 mM Tris/Trizma® HCl (pH 7.4) containing either 0.1% or 1% (2.7 or 27 mM) cetrimide was placed in the anodal compartment and a current of 0.15 mA/cm^2 was applied for either 8 or 24 h.

Pretreatment

Vapreotide iontophoresis was preceded by the iontophoretic delivery of either propranolol or cetrimide:

A: A solution of 40 mM propranolol or 0.1% cetrimide in 25 mM Tris/Trizma® HCl (pH 7.4) normal saline was placed in the anodal compartment and a current of 0.3 mA/cm^2 was applied for 8 h.

B: Subsequently, 1 ml of 4.4 mM vapreotide in 20 mM Tris/Trizma® HCl (pH 7.4) was placed in the donor compartment and a current of 0.15 mA/cm^2 was applied for 24 h using a salt bridge.

Post-Treatment

Solutions of $CaCl_2$, NaCl, propranolol, and cetrimide were tested with respect to their capacity to release vapreotide bound to, and accumulated within, dermatomed skin.

These experiments also comprised two parts:

A: 1 ml of vapreotide (3 mM) in 20 mM Tris/Trizma® HCl (pH 7.4) was placed in the donor compartment and a current of 0.15 mA/cm^2 was applied for 24 h using a salt bridge.

B: Solutions of either 100 mM $CaCl_2$, 100 mM NaCl, 40 mM propranolol, or 0.1% cetrimide in 25 mM Tris/Trizma® HCl (pH 7.4) normal saline were added to the anodal compartment and a current of 0.3 mA/cm^2 was applied for 8 h.

For each pre- and post-treatment experiment, the receptor compartment was replaced between phase A and phase B.

Data Treatment

The results were derived from at least triplicate experiments conducted with skin samples originating from different pig ears. Outliers, determined using the Grubbs test, were discarded. When two sets of data where compared, Student *t*-tests were performed. The level of statistical significance was fixed at $p < 0.05$.

RESULTS AND DISCUSSION

Skin Metabolism of Vapreotide

Cutaneous metabolism has been widely investigated (16–18) and several studies into transdermal peptide delivery have reported the presence of proteolytic activity in the skin (9,19–21). With the concentration tested, only 39 ± 18% of the vapreotide in contact with the dermal face of dermatomed skin samples was found to be intact after 8 h of iontophoresis. Similarly, only 32 ± 11% of the peptide remained intact after exposure to the interior epidermal surface. The levels of degradation observed following exposure to either dermatomed skin or isolated epidermis were not significantly different.

Although the presence of proteolytic enzymes in the skin is generally accepted, their exact location and distribution in the distinct tissue layers of the skin remains unknown. Although the epidermis is claimed to be the major site of drug degradation, activity has also been ascribed to the dermis (17). Skin contact studies with vapreotide suggest that both epidermal and dermal tissues possess some form of proteolytic activity. Moreover, the results also demonstrate that the enzymes located in the epidermis are resistant to the heat separation treatment used to isolate the epidermal membrane.

Nevertheless, it is difficult to extrapolate these *in vitro* results to the eventual situation *in vivo* (particularly in humans), notably owing to major interspecies differences in the structure and function of such enzymes. In addition, the residence time of the peptide in the enzymatic barrier is likely to be significantly reduced *in vivo* as molecules reaching the epidermal–dermal junction are taken up rapidly by the skin's microcirculation. Although enzymatic activity is expected to be higher in viable tissues, it can be argued that *ex vivo* skin preparation procedures might result in increased release of intracellular enzymes. However, the skin tissue used in these permeation experiments was frozen prior to use, a process reported to reduce metabolic activity of skin samples (22). Thus, *in vitro*/*in vivo* correlations for labile peptides are complicated by the interplay of these opposing factors.

Vapreotide Transport

Under the same iontophoretic conditions, significantly more (~10-fold) vapreotide was transported across the epidermis (~10 μg) than across dermatomed skin (~1 μg) when using the experimental set-up employing a salt-bridge (Fig. 2). It is generally accepted that the remarkable barrier function of the skin is primarily located in the outermost layer of the epidermis, the stratum corneum, and that, *in vivo*, substances are taken up by the capillary network adjacent to the epidermal–dermal junction, after diffusing on the order of 100 μm through the membrane. In separate studies (data not shown), there was no difference between the amount of vapreotide transported across tape-stripped and intact dermatomed skin, suggesting that the stratum corneum was not the rate-limiting barrier to transport at least under *in vitro* conditions. The use of dermatomed skin (~800 μm in thickness) in these *in vitro* experiments introduces an additional mass of dermal tissue, which acts as a "pseudo-receptor" compartment, notably for compounds such as vapreotide, which display a high skin affinity. Therefore, the presence of dermis provides an artifactual reservoir for the drug that has already crossed the epidermis, and which, *in vivo*, would be taken up by the capillaries just below the epidermal–dermal junction. Thus, epidermis, which is a well-established model for transdermal drug delivery, appears to be more appropriate than dermatomed skin for transdermal vapreotide transport studies. The fate of a peptide reservoir *in vivo*, if indeed it exists, remains to be studied.

Vapreotide Accumulation in the Skin

Despite the inefficient transfer of vapreotide across dermatomed skin, significant amounts of the peptide (80–300 μg) were recovered from the skin samples after

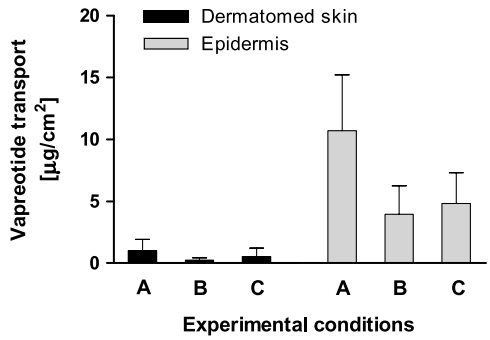

Fig. 2. Cumulative amount of vapreotide measured in the receptor compartment after 8 h of iontophoresis at 0.15 mA/cm^2 across epidermis and dermatomed skin as a function of the experimental set-up. (A: with salt bridge, B: without salt bridge, C: without salt bridge; with BSA). There were statistically significant differences between epidermis and dermatomed skin in each corresponding experimental condition ($p < 0.05$).

Table I. Vapreotide Accumulation in the Skin After Iontophoretic Current Application for 8–24 h Using Formulations Containing 1.5–4.4 mM Vapreotide

Experimental condition	Amount of vapreotide in the skin (μg)
24 h iontophoresis at 0.15 mA/cm^2, 4.4 mM donor concentration	210 ± 10
8 h iontophoresis at 0.15 mA/cm^2, 3 mM donor concentration	80 ± 20
18 h iontophoresis at 0.5 mA/cm^2, 1.5 mM donor concentration	300 ± 60

Experiments were conducted using the experimental set-up shown in Fig. 1B.

iontophoresis (Table 1). These peptide accumulation data further attest to the suspected strong association between molecules possessing a positive charge, in close proximity to a lipophilic surface, and the skin. In addition to these specific interactions between the peptide and structures present in the skin, the accumulation may also be the result of peptide–peptide interactions, which result in vapreotide aggregation and eventually in peptide deposition in the transport pathways.

Effect of Vapreotide Transport on Skin Permselectivity

Previous studies investigating iontophoretic transport across hairless mouse, rabbit, porcine, and human skin have confirmed the existence of skin permselectivity, a phenomenon that gives rise to electroosmosis and contributes to the iontophoretic transport of neutral and cationic species (23–25). Moreover, the ability of certain lipophilic, peptidic and non-peptidic cations to inhibit the convective solvent flow has also been established (10,11,26). The magnitude and significance of this inhibition depend on the skin type and the physicochemical properties of the molecule. The effect of current density on the inhibition of electroosmosis by vapreotide is illustrated in Fig. 3. The inhibitory capacity of vapreotide is strongly dependent on the applied current density, increasing the inhibition factor by almost 50-fold at 0.5 mA/cm^2. As current density is increased, more charge has to be transported across the skin; this is partly carried by the peptide, which is driven into the membrane in greater amounts, leading to a more extensive neutralization of the skin's negative charge and a more pronounced inhibition of electroosmosis.

Effect of Reducing Competing Ions in the Formulation

Because Ag/AgCl electrodes need chloride ions for anodal electrochemistry, the anodal compartment must contain a supply of such ions derived from either the active agent (e.g., hydrochloride salts) or from an external source (e.g., NaCl), in which case the total number of cations in the anodal formulation is greatly increased. These cations compete with the positively charged drug as charge carriers. The use of a salt bridge enables fewer competing cations (~20 mM) to be incorporated in the donor compartment, increasing vapreotide transport efficacy, as indicated in Fig. 2. To confirm that the ionic strength and not the donor pH, which also varied between the two experimental set-ups, was responsible for the modified transport of vapreotide, the effect of pH was also investigated. A control experiment using the "salt bridge" set-up was performed at pH 5.5 and revealed that there was no significant difference in the quantity of vapreotide transported (11.9 ± 3.8 μg/cm^2 at pH 5.5 *vs.* 10.7 ± 4.5 μg/cm^2 at pH 7.4).

Figure 4 indicates that the inhibition factor is increased twofold in the presence of the salt bridge. As discussed earlier, vapreotide transport increases with use of a salt bridge; hence, more peptide reaches the skin membrane, resulting in a more complete neutralization of skin charge and, as a result, greater reduction in electroosmotic flow. However, this increased inhibition is not sufficient to outweigh the increased transport resulting from the reduced ion competition (Fig. 2). Finally, the degree of electroosmotic inhibition was also independent of the skin preparation and experimental conditions employed (Fig. 4).

In addition to increasing the proportion of charge carried by the peptide, salt bridges allow the isolation of the peptide from the electrode compartment and have the

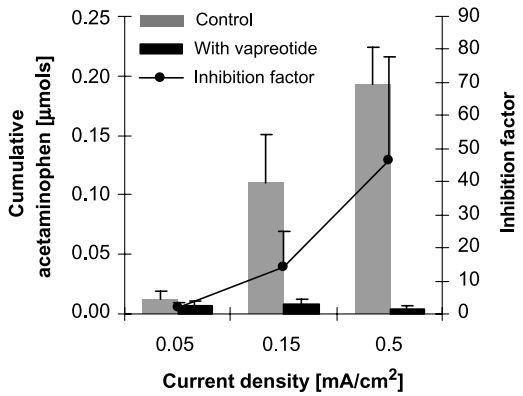

Fig. 3. Cumulative amounts of acetaminophen delivered across porcine ear skin during 8 h of transdermal iontophoresis at 0.05, 0.15, and 0.5 mA/cm^2 in the presence and absence of vapreotide. The inhibition factor is calculated according to Eq. (1).

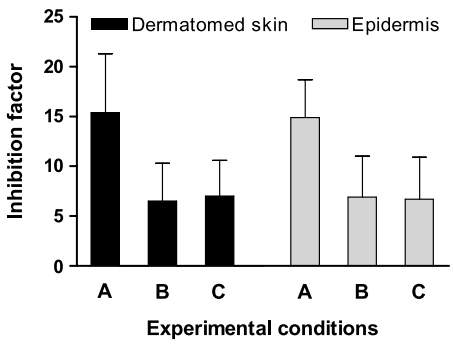

Fig. 4. The electroosmotic inhibition factor after 8 h of iontophoresis at 0.15 mA/cm^2 across epidermis and dermatomed skin as a function of the experimental set-up. (A: with salt bridge, B: without salt bridge, C: without salt bridge; with BSA). There were statistically significant differences between the IFs observed with and without salt bridges for both epidermal and dermatomed skin samples ($p < 0.05$).

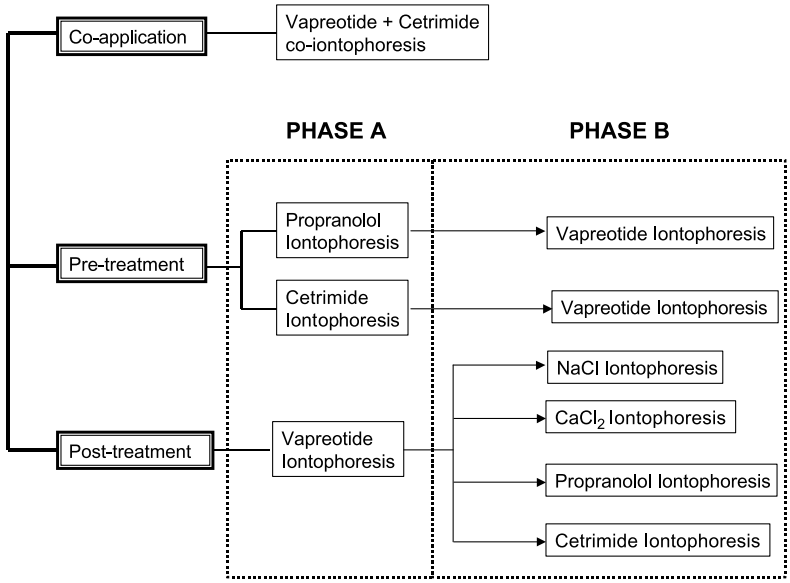

Fig. 5. Experimental strategies investigated to reduce the degree of vapreotide fixation to the skin.

advantage of preventing interactions between the electrode and the drug. Patch-based iontophoretic systems employing the same principles have been developed in which the electrode is separated from the drug reservoir by a size exclusion membrane (27).

Modifying Interactions Between Vapreotide and the Skin

Given the substantial amounts of peptide measured in the skin, the liberation and subsequent transport of even a small fraction of this bound peptide would result in the delivery of significant amounts of drug. A number of different approaches, as shown in Fig. 5, were employed to modulate the impact of peptide–skin interactions on vapreotide transport.

The first approach involved co-iontophoresis of the cationic surfactant, cetrimide. The results in Fig. 6A show that co-iontophoresis of 0.1% cetrimide led to a twofold reduction in the amount of peptide recovered from the skin. As shown in Fig. 6B, increasing the cetrimide concentration from 0.1% to 1% produced a greater effect. Although it is tempting to attribute this to an interaction of the cationic cetrimide with putative binding sites, the observation that peptide transport *through* the membrane was not increased suggests that the surfactant itself acted as a competing charge carrier.

To exclude the risk of competition, subsequent studies employed pre- and post-treatment of the skin wherein cetrimide and other species known to bind to the membrane, for example, Ca^{2+} and propranolol, were iontophoresed either prior to or after vapreotide iontophoresis. It has been proposed that the lower transport number of Ca^{2+} as compared to Na^{+} is attributable to its superior interaction with negatively charged binding sites in the skin (28). Likewise, the iontophoresis of propranolol (40 mM) has been shown to reduce the electroosmotic transport of mannitol across porcine ear skin (26). Pre-iontophoretic treatment with either cetrimide or propranolol was thus attempted to saturate the putative binding sites, and hence prevent the interaction of vapreotide with the skin. Vapreotide transport following these pretreatments was not statistically different from the control ($p < 0.05$). Previous reports showed that cetrimide pretreatment reduced iontophoretic delivery of propranolol and this was attributed to a neutralisation of skin charge (29). Based on the acetaminophen flux measured in our studies, inclusion of cetrimide in the peptide formulation produced an approximately twofold increase in electroosmotic inhibition as compared to iontophoresis of vapreotide alone;

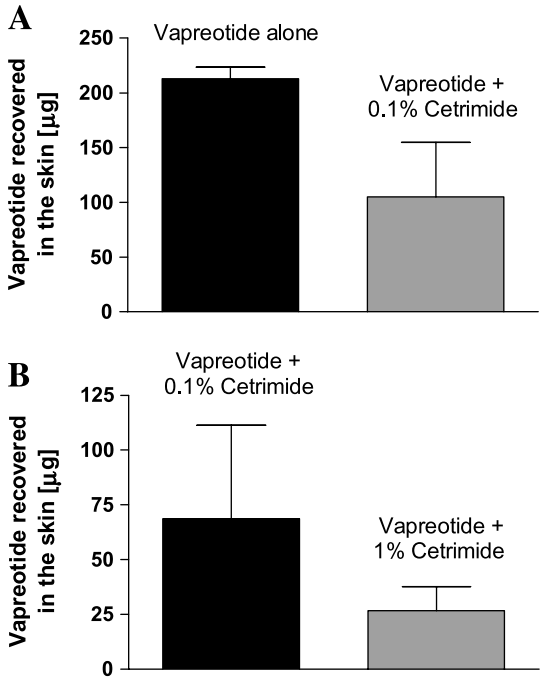

Fig. 6. Effect of co-iontophoresis of cetrimide on the amount of vapreotide bound to the skin. (A) Co-iontophoresis of 0.1% surfactant with 4.4 mM vapreotide for 24 h at 0.15 mA/cm^2. (B) Co-iontophoresis of 0.1% and 1% surfactant with 4.4 mM vapreotide for 8 h at 0.15 mA/cm^2.

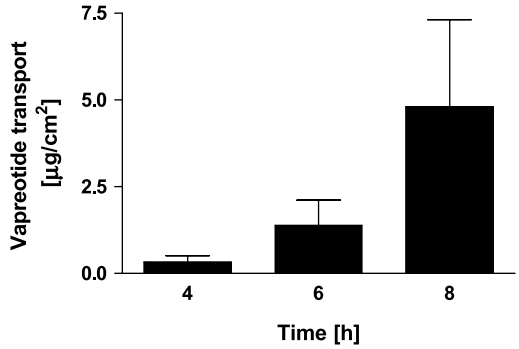

Fig. 7. Cumulative amount of vapreotide present in the receptor compartment after 4, 6, and 8 h of iontophoretic delivery at 0.15 mA/cm^2 across porcine epidermis.

furthermore, it may also accumulate in the transport pathways impeding vapreotide passage. Post-iontophoretic treatment methods were investigated with the aim of displacing bound peptide from the skin. However, almost negligible amounts of the peptide (<20 ng/cm^2) were released after 8 h of post-iontophoretic treatment with the different cationic species tested.

Taken together, these results indicate that the strategies employed to modify skin–vapreotide interactions in order to improve peptide delivery were unsuccessful.

Can Therapeutic Amounts of Vapreotide be Delivered?

To compare our *in vitro* vapreotide transport studies with the earlier *in vivo* investigation conducted by Lau *et al.* into the transdermal iontophoresis of octreotide (13), the final set of permeation experiments were conducted without a salt bridge and with BSA in the receptor compartment. The amounts of vapreotide reaching the receptor compartment after 4, 6, and 8 h of iontophoretic current application (0.15 mA/cm^2) are shown in Fig. 7. These cumulative quantities were used to estimate the vapreotide flux at 7 h (1.7 µg/cm^2 per hour). Since the experimental conditions chosen for the *in vitro* study were similar to those used by Lau *et al.* for the iontophoretic delivery of octreotide acetate in the rabbit, a comparison of the transport of these two somatostatin analogues is feasible. After 8 h of iontophoresis at 0.15 mA/cm^2, the plasma concentrations of octreotide were 0.85 and 1.7 ng/ml with donor concentrations of 2.5 and 5 mg/ml, respectively (13). Based on our vapreotide data (donor concentration of 3 mM $\cong$ 3.9 mg/ml) and the relationship equating flux to plasma concentration (steady-state flux $\times$ area = clearance [plasma]), it is possible to estimate the plasma concentration of vapreotide if it were delivered to the rabbit under similar conditions (assuming the same clearance of 2.3 L/h); the estimated value is 0.7 ng/ml, suggesting a slightly lower transport for vapreotide than for octreotide. Aside from the subtle differences in amino acid sequence, this might be due in part to (1) the different skin models used in the two studies (rabbit skin is recognized as being more permeable than porcine skin) and (2) the aforementioned differences in metabolic activities (both inter-species and *in vitro*/*in vivo*).

Given a total vapreotide clearance of 16.7 L/h in humans (personal communication), an input rate of ~25 µg/h must be achieved in order to maintain a therapeutic level of 1.5 ng/ml. In view of the experimental *in vitro* flux (1.7 µg/cm^2 per hour), it follows that a patch of ~15 cm^2 could achieve the desired delivery kinetics. Moreover, given the modest experimental conditions used in this preliminary study, the enhancement of vapreotide delivery by further fine-tuning of the formulation and current profile is a realizable goal. For example, the results obtained with a salt bridge system clearly demonstrate that vapreotide transport can be enhanced by reducing the number of extraneous ions. Hence, based on the data presented here, the transdermal iontophoretic delivery of therapeutic amounts of vapreotide certainly appears to be feasible.

CONCLUSIONS

As with the luteinizing hormone releasing hormone analogues, nafarelin and leuprolide, and other nonpeptidic compounds that possess a structural motif characterized by the close proximity of charge and lipophilicity (e.g., propranolol and quinine), vapreotide has been shown to interact with the skin membrane. The degree of electroosmotic inhibition increased with increasing current density. As a consequence of this interaction, and perhaps the aggregation of the peptide, large quantities of vapreotide were found in the skin. The pretreatment strategies to saturate the skin binding sites and the post-treatment methods to displace bound vapreotide proved ineffective. It was also shown that significantly greater amounts of peptide were transported across heat-separated epidermis than across dermatomed skin. Vapreotide was susceptible to a significant degree of metabolism (~60–70%) when placed in contact with the interior surface of heat-separated epidermis or dermatomed skin. Given its susceptibility to enzymatic degradation, and its capacity to shut down electroosmotic solvent flow, vapreotide may not appear as an ideal candidate for transdermal iontophoresis. Nevertheless, analysis of the cumulative amounts permeated across the epidermis and the human pharmacokinetics suggests that iontophoretic delivery might be an effective alternative to the conventional parenteral administration of vapreotide.

REFERENCES

1. P. Calès, C. Masliah, D. Lebrec, B. Bernard, and P.-P. Garnier. Early administration of vapreotide for variceal bleeding in patients with cirrhosis. *N. Engl. J. Med.* **344**:23–28 (2001).
2. E. Köhler, M. Duberow-Drewe, J. Drewe, G. Ribes, M. M. Loubatieres-Mariani, N. Mazer, K. Gyr, and C. Beglinger. Absorption of an aqueous solution of a new synthetic somatostatin analogue administered to man by gavage. *Eur. J. Clin. Pharmacol.* **33**:167–171 (1987).
3. C. Invitti, L. M. Fatti, F. Cavagnini, H. Orskov, L. Porcu, and M. G. Camboni. Octreotide nasal power in acromeglic patients: a dose-range and tolerability study. *J. Endocrinol. Invest.* **17**:A41 (1994).
4. R. H. Guy. Current status and future prospects of transdermal drug delivery. *Pharm. Res.* **13**:1765–1769 (1996).
5. Y. N. Kalia, A. Naik, J. Garrison, and R. H. Guy. Iontophoretic drug delivery. *Adv. Drug Deliv. Rev.* **56**:619–658 (2004).
6. R. H. Guy, Y. N. Kalia, M. B. Delgado-Charro, V. Merino, A. Lopez, and D. Marro. Iontophoresis: electrorepulsion and electroosmosis. *J. Control. Release* **64**:129–132 (2000).
7. J. Hirvonen and R. H. Guy. Iontophoretic delivery across the

skin: electroosmosis and its modulation by drug substances. *Pharm. Res.* **14**:1258–1263 (1997).
8. M. B. Delgado-Charro and R. H. Guy. Characterisation of convective solvent flow during iontophoresis. *Pharm. Res.* **11**:929–935 (1994).
9. M. B. Delgado-Charro and R. H. Guy. Iontophoretic delivery of nafarelin across the skin. *Int. J. Pharm.* **117**:165–172 (1995).
10. J. Hirvonen, Y. N. Kalia, and R. H. Guy. Transdermal delivery of peptides by iontophoresis. *Nat. Biotechnol.* **14**:1710–1713 (1996).
11. J. Hirvonen and R. H. Guy. Transdermal iontophoresis: modulation of electroosmosis by polypeptides. *J. Control. Release* **50**:283–289 (1998).
12. A. J. Hoogstraate, V. Srinivasan, S. M. Sims, and W. I. Higuchi. Iontophoretic enhancement of peptides: behaviour of leuprolide *versus* model permeants. *J. Control. Release* **31**:41–47 (1994).
13. D. T. W. Lau, J. W. Sharkey, L. Petryk, F. A. Mancuso, Z. Yu, and F. L. S. Tse. Effect of current magnitude and drug concentration on iontophoretic delivery of octreotide acetate (Sandostatin) in the rabbit. *Pharm. Res.* **11**:1742–1746 (1994).
14. S. Santoyo, A. Arellano, P. Ygartua, and C. Martin. Penetration enhancer effects on the *in vitro* percutaneous absorption of piroxicam through rat skin. *Int. J. Pharm.* **117**:219–224 (1995).
15. P. Glikfeld, C. Cullander, R. S. Hinz, and R. H. Guy. A new system for *in vitro* studies of iontophoresis. *Pharm. Res.* **5**:443–446 (1988).
16. S. W. Collier, J. E. Storm, and R. L. Bronaugh. Cutaneous metabolism. In R. L. Bronaugh and H. I. Maibach (eds.), *In Vitro Percutaneous Absorption: Principles, Fundamentals, and Applications*, CRC Press, Boca Raton, FL, 1991, pp. 67–83.
17. I. Steinsträsser and H. P. Merkle. Dermal metabolism of topically applied drugs: pathways and models reconsidered. *Pharm. Acta Helv.* **70**:3–24 (1995).
18. J. E. Fräki, G. S. Lazarus, and V. K. Hopsu-Havu. Protein catabolism in the skin. In L. A. Goldsmith (ed.), *Biochemistry and Physiology of the Skin*, Oxford University Press, New York, 1983, pp. 338–362.
19. L. L. Miller, C. J. Kolaskie, G. A. Smith, and J. Rivier. Transdermal iontophoresis of gonadotropin releasing hormone (LHRH) and two analogues. *J. Pharm. Sci.* **79**:490–493 (1990).
20. S. D. Roy and J. S. de Groot. Percutaneous absorption of nafarelin acetate, an LHRH analog, through human cadaver skin and monkey skin. *Int. J. Pharm.* **110**:137–145 (1994).
21. A. K. Banga, M. Katakam, and R. Mitra. Transdermal iontophoretic delivery and degradation of vasopressin across human cadaver skin. *Int. J. Pharm.* **166**:211–216 (1995).
22. N. Higo, R. S. Hinz, D. T. W. Lau, L. Z. Benet, and R. H. Guy. Cutaneous metabolism of nitroglycerin. II. Effect of skin conditions and penetration enhancement. *Pharm. Res.* **9**:299–302 (1992).
23. A. Luzardo-Alvarez, M. Rodriguez-Fernandez, J. Blanco-Mendez, and M. B. Delgado-Charro. Iontophoretic permselectivity of mammalian skin: characterization of hairless mouse and porcine membrane models. *Pharm. Res.* **15**:984–987 (1998).
24. D. Marro, R. H. Guy, and M. B. Delgado-Charro. Characterization of the iontophoretic permselectivity properties of human and pig skin. *J. Control. Release* **70**:213–217 (2001).
25. S. Nicoli, M. Cappellazzi, P. Colombo, and P. Santi. Characterization of the permselective properties of rabbit skin during transdermal iontophoresis. *J. Pharm. Sci.* **92**:1482–1488 (2003).
26. D. Marro, Y. N. Kalia, M. B. Delgado-Charro, and R. H. Guy. Contributions of electromigration and electroosmosis to iontophoretic drug delivery. *Pharm. Res.* **18**:1701–1708 (2001).
27. S. R. Patel, H. Zhong, A. Sharma, and Y. N. Kalia. Non-invasive controlled delivery of zolmitriptan *in vivo* by transdermal iontophoresis. *Cephalgia* **24**:793 (2004).
28. R. R. Burnette. Iontophoresis. In J. Hadgraft and R. H. Guy (eds.), *Transdermal Drug Delivery-Developmental Issues and Research Initiatives*, Marcel Dekker, New York, 1989, pp. 247–291.
29. S. Chesnoy, D. Durant, J. Doucet, and G. Couarraze. Structural parameters involved in the permeation of propranolol HCl by iontophoresis and enhancers. *J. Control. Release* **58**:163–175 (1999).